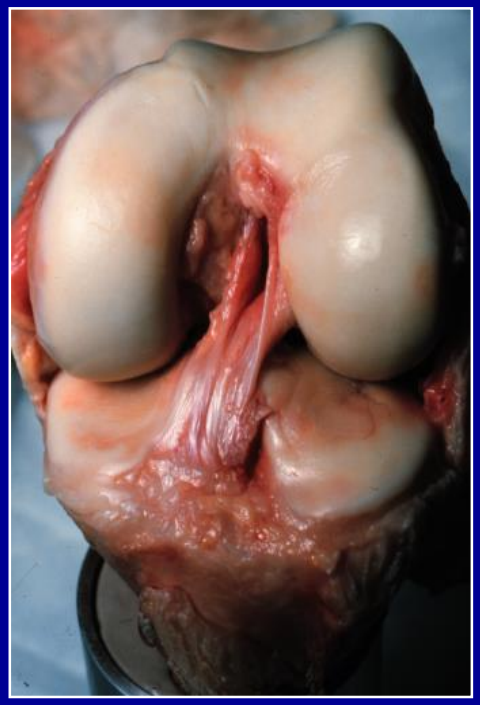
ΕΜΒΙΟΜΗΧΑΝΙΚΗ

Εφαρμογές μηχανικής στο επίπεδο κυττάρου και ιστού

Articular cartilage

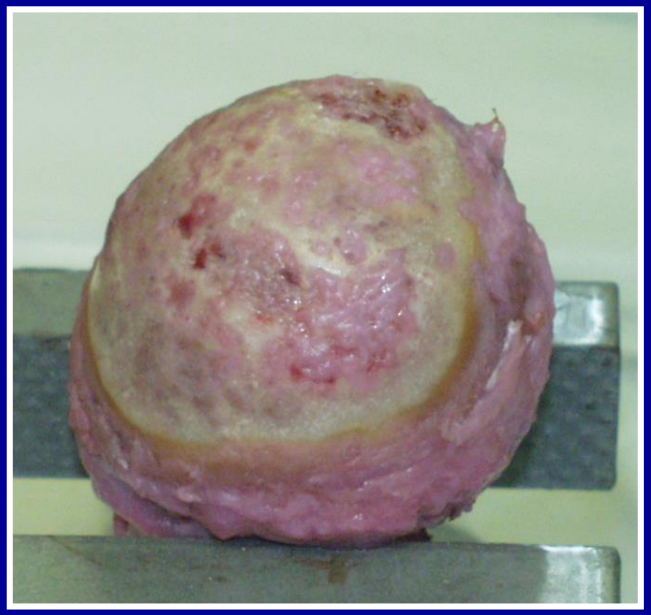


- Avascular, aneural
- Load-bearing surface
- Transfer loads with minimal wear and friction

OUR GOAL:

To study the etiology, prevention, and treatment of arthritis, a painful disease that leads to the destruction of the joints of the body

Facts about Arthritis*

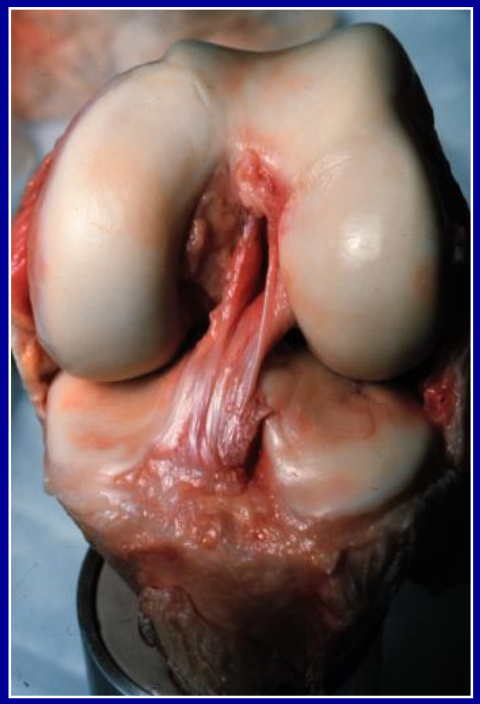


- The leading cause of disability in the US
- Affects over 43 million Americans
- Costs the US economy $65 Billions/year

- The most common type of Arthritis is **OSTEOARTHITIS**
- Usually begins after age 45
- Causes symptoms in 1 of every 3 individuals over age 60
- Risk Factors include Age, Obesity & Abnormal mechanical load

* CDC, 1996; Praemer et al., 1999

Articular cartilage composition



SURFACE ZONE

MIDDLE ZONE

DEEP ZONE

~80% Water

~15% Collagen (type II)

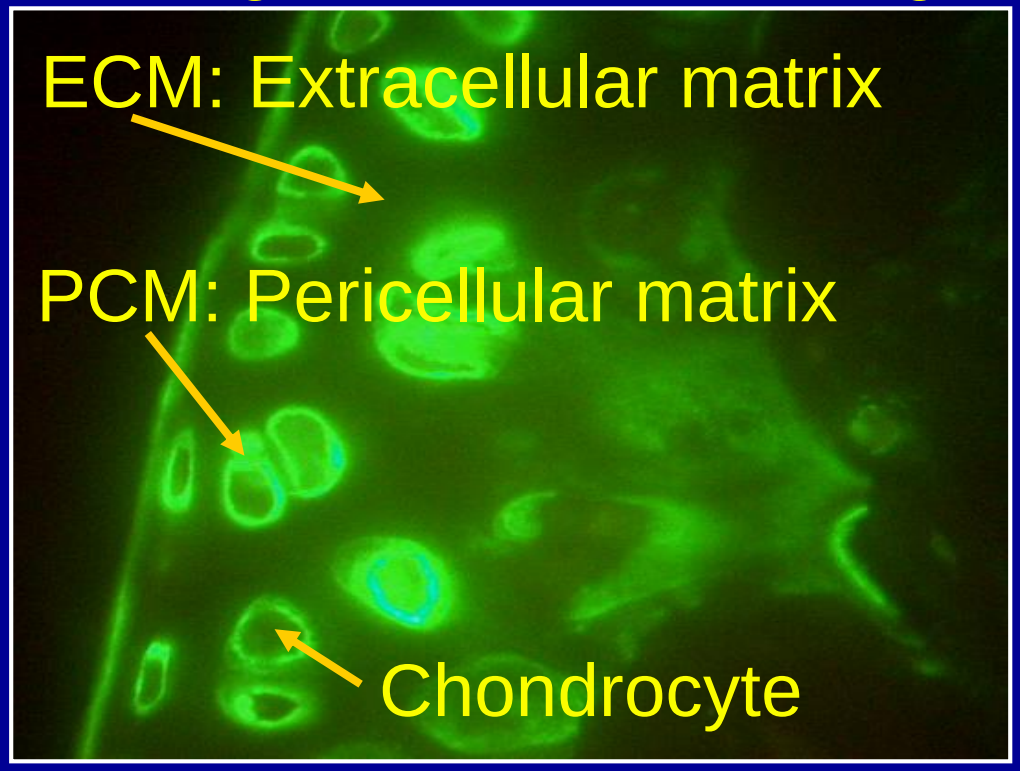
~5% Proteoglycans

~1% Chondrocyte

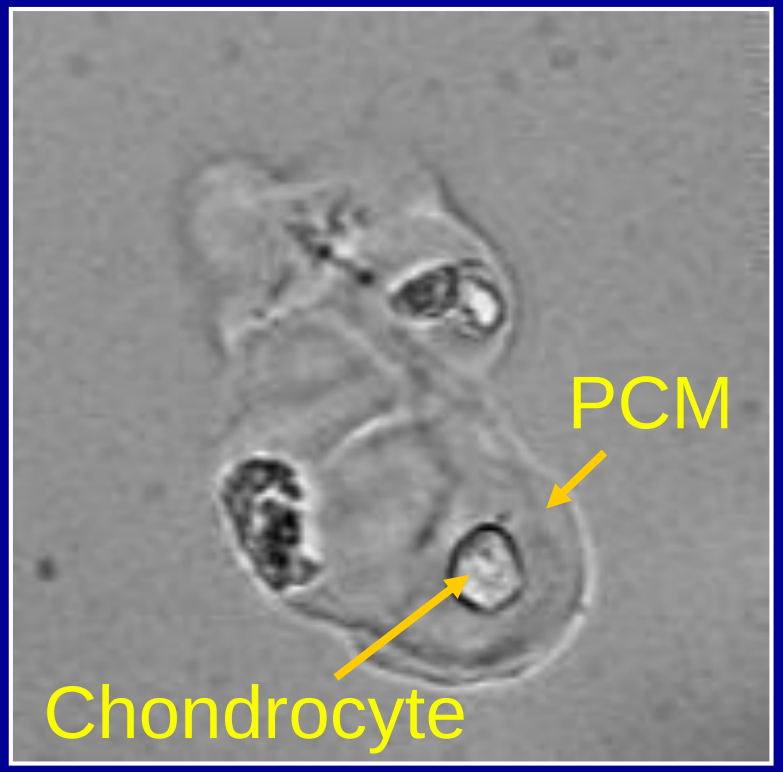
Other proteins, lipids

Chondron

Collagen VI immunostaining



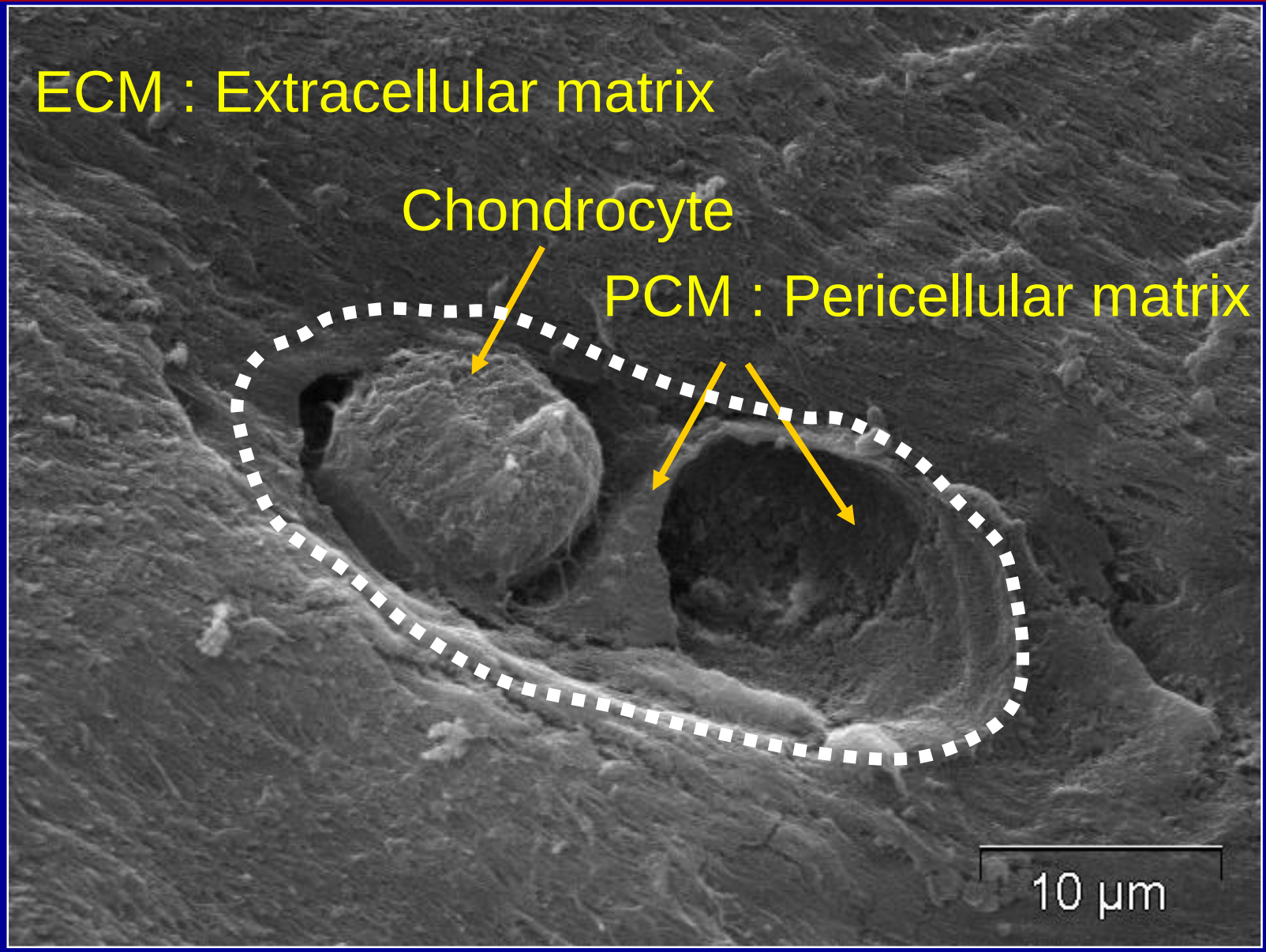
Isolated chondron



Pericellular Matrix (PCM):

- Similar composition to the ECM
- EXCLUSIVE PRESENCE OF TYPE VI COLLAGEN

Chondron = CHONDROCYTE +PCM



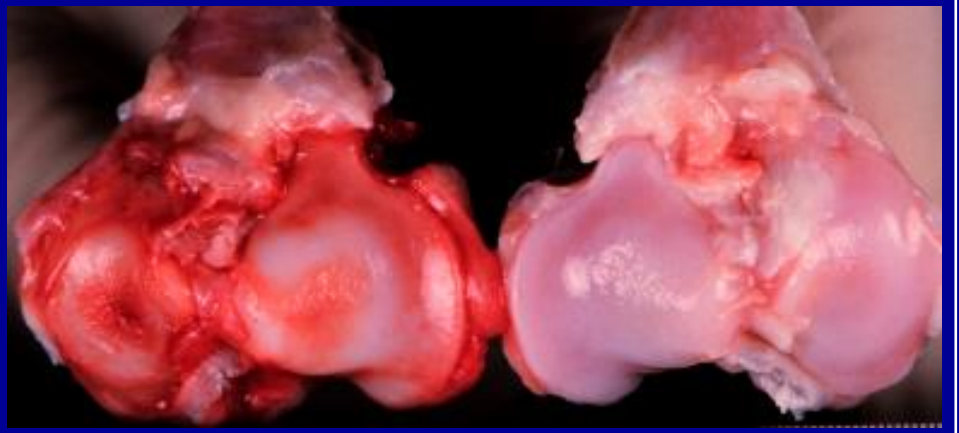
What is the role of the PCM in normal and osteoarthritic cartilage?

The role of PCM is speculative.

It completely surrounds the chondrocyte and thus mediates all the biological and mechanical signals that the chondrocyte experiences

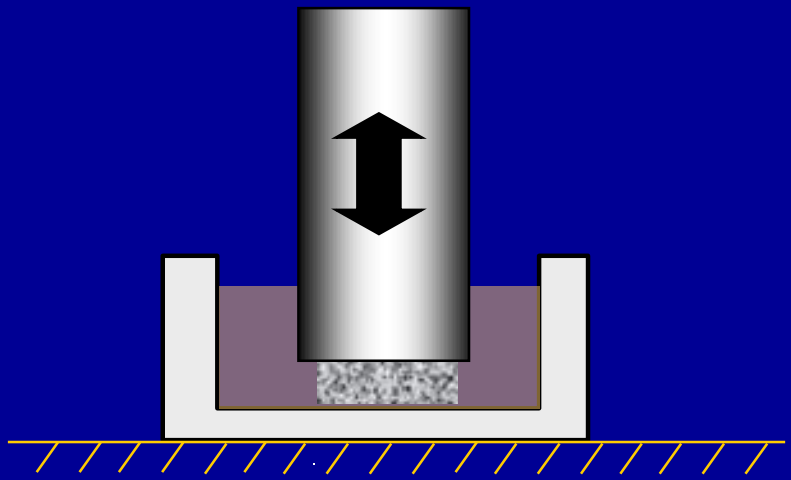
Mechanical environment of chondrocytes is important in cartilage homeostasis

In vivo studies



Abnormal mechanical loading results in osteoarthritic (OA) changes in articular cartilage

In vitro studies



Dynamic compression:
Anabolic effects
Static compression:
Catabolic effects

Freeman et al. 1994, Setton et al. 1994, van Campen et al. 1987, Buschmann et al. 1999, Regan et al. 1999

1. Υπολογισμός μηχανικών ιδιοτήτων χόνδρου
2. Υπολογισμός του τασικού πεδίου του κυττάρου σε φυσιολογικό και αρθριτικό χόνδρο
3. Μοντελοποίηση λειτουργίας κυττάρου
4. Εκτίμηση βέλτιστης φαρμακευτικής παρέμβασης μέσω μοντελοποίησης. Υπολογισμός δραστικότητας μέσω προσδιορισμού μέτρου ελαστικότητας

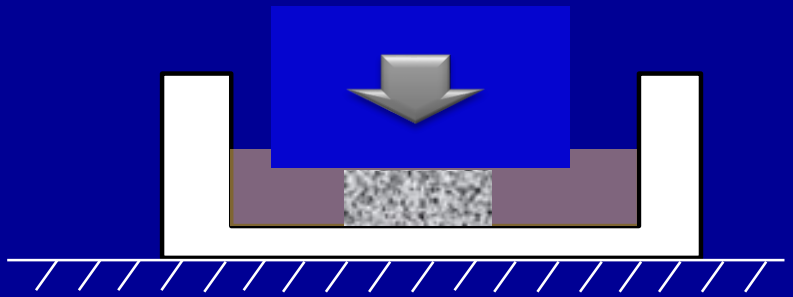
Measuring cartilage degeneration

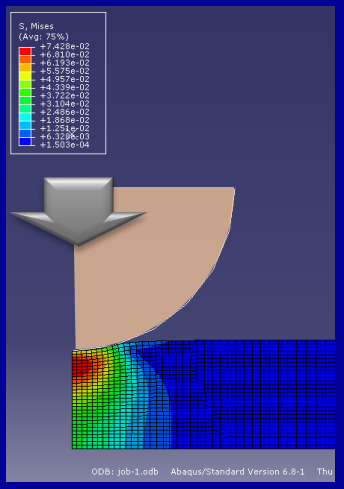
Quantify cartilage degeneration via mechanical properties

Elasticity (Young's modulus, E)

Permeability (k)

Experimental set up

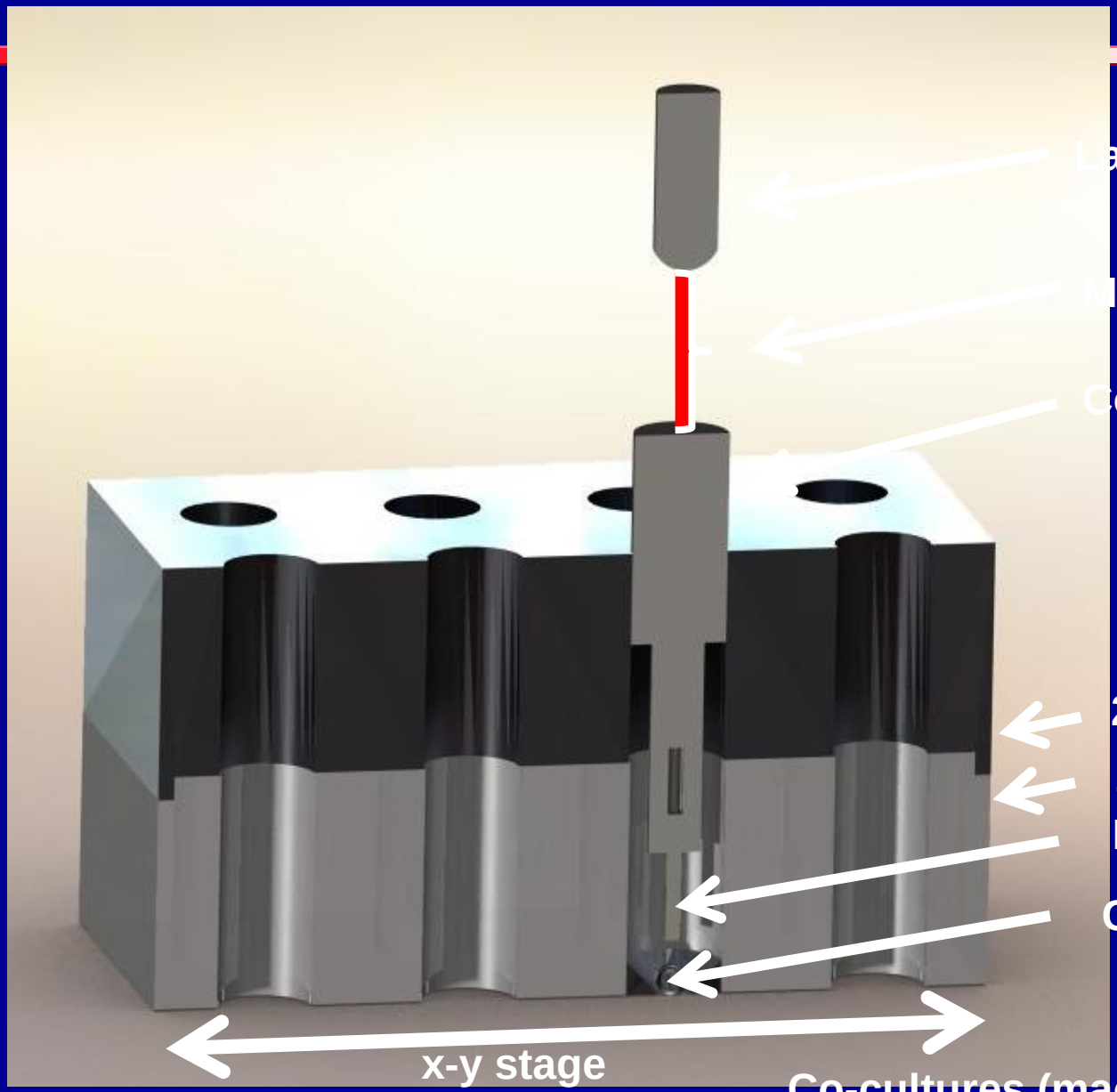




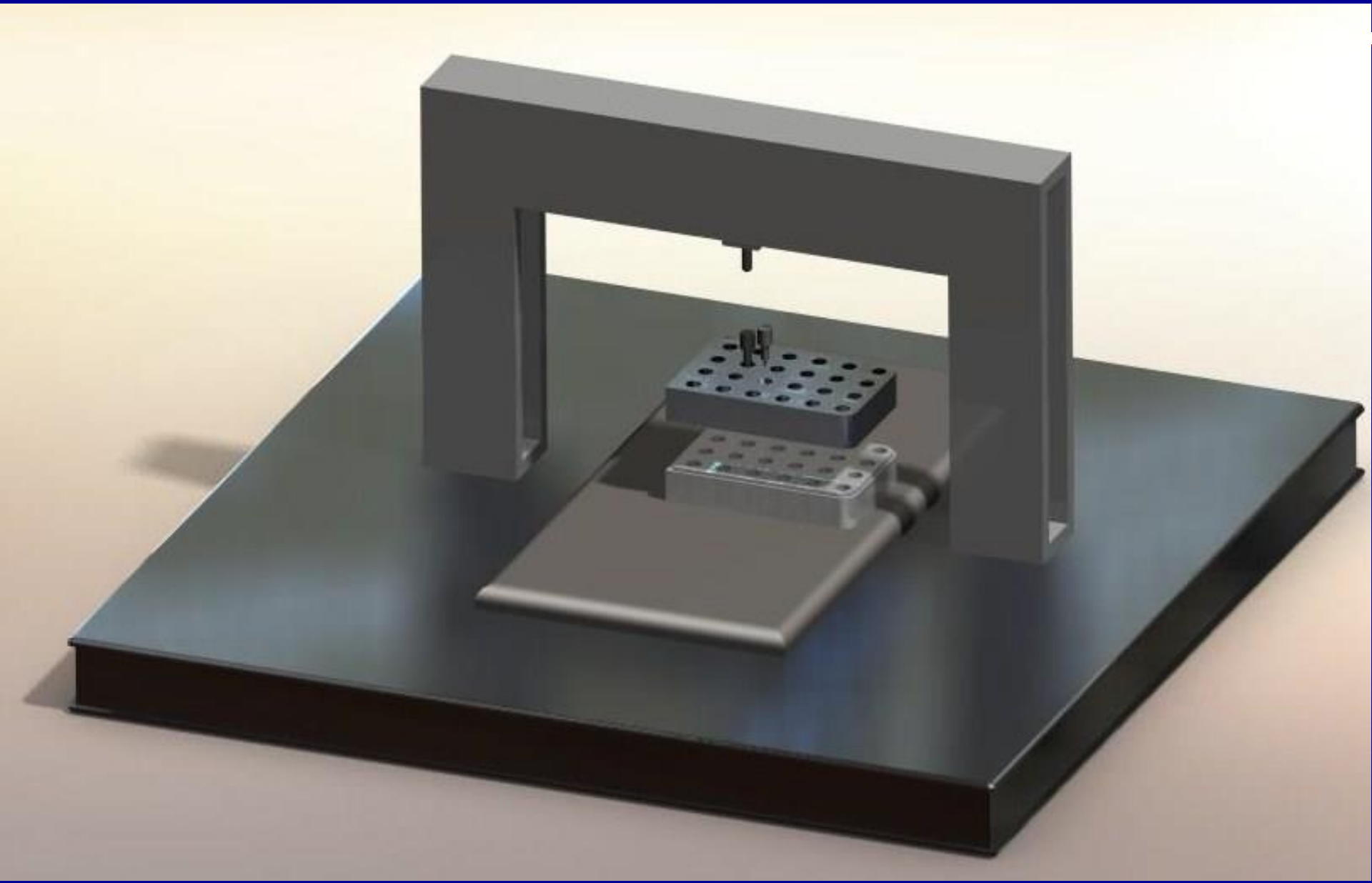
Biphasic FEM

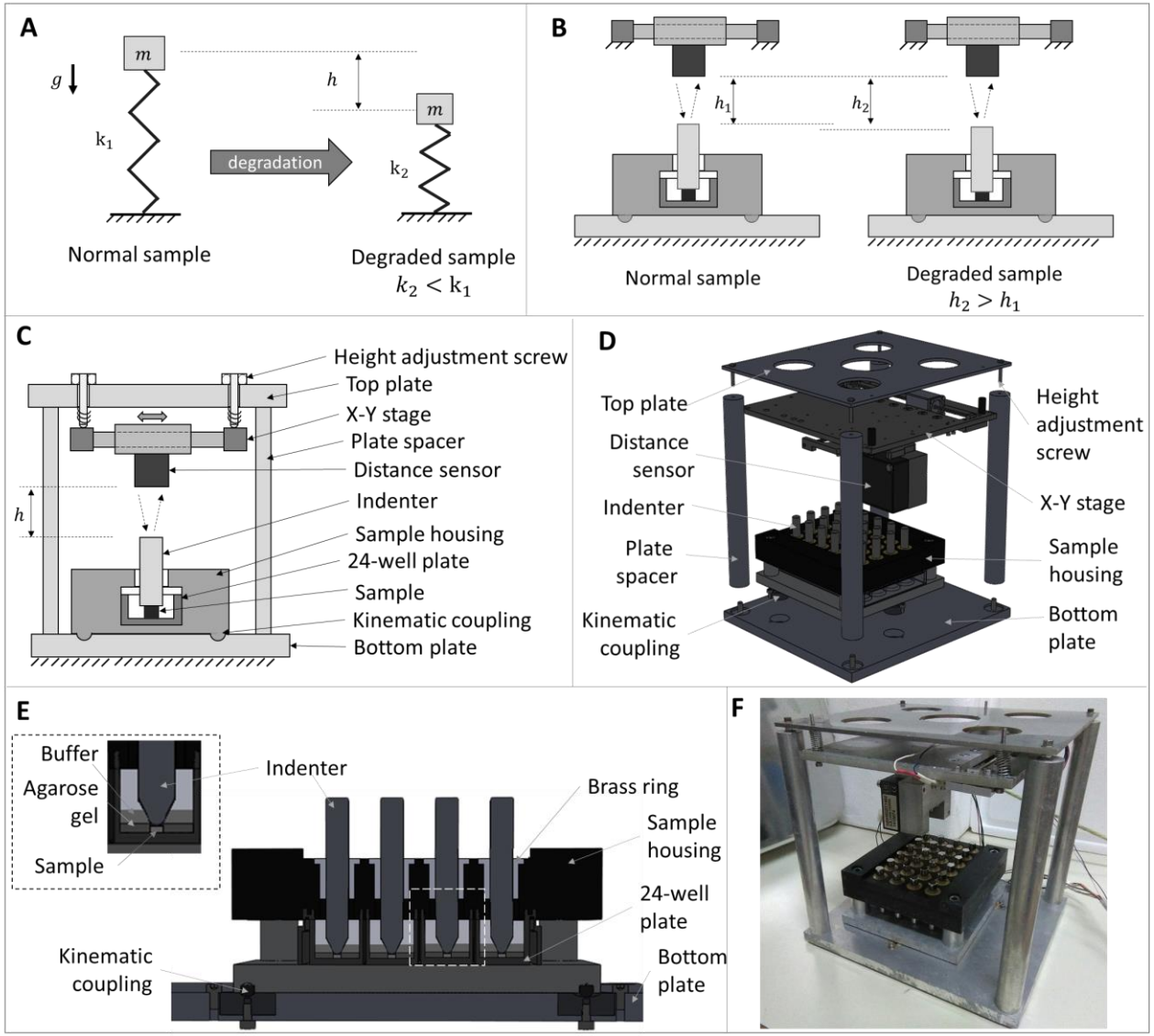
Alexopoulos et al *J Biomech Eng* (2003), Alexopoulos et al *Acta Biom.* (2005), Alexopoulos et al J *Biomech* (2003)

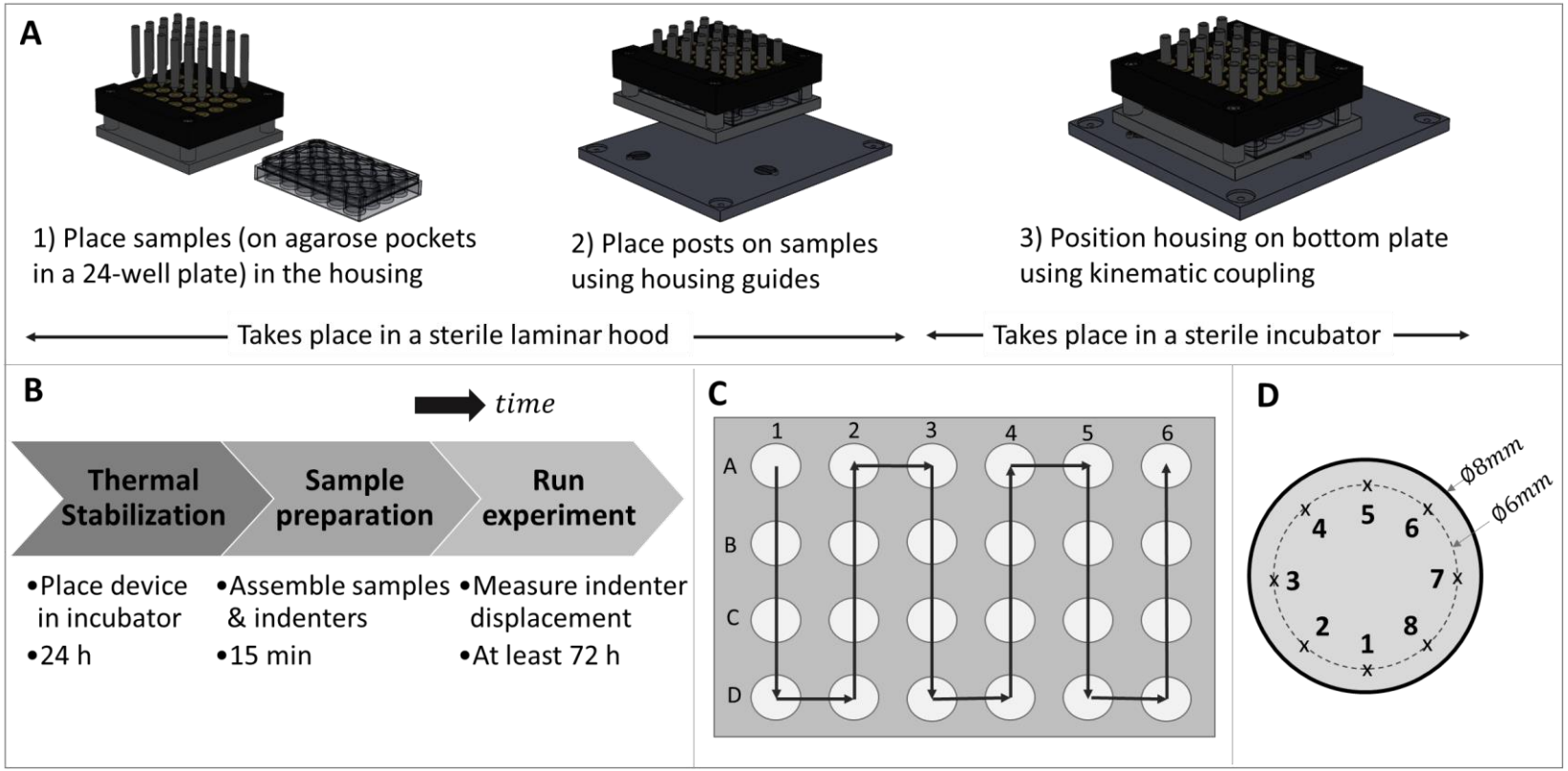
In-vitro system for real-time monitoring of cartilage degeneration

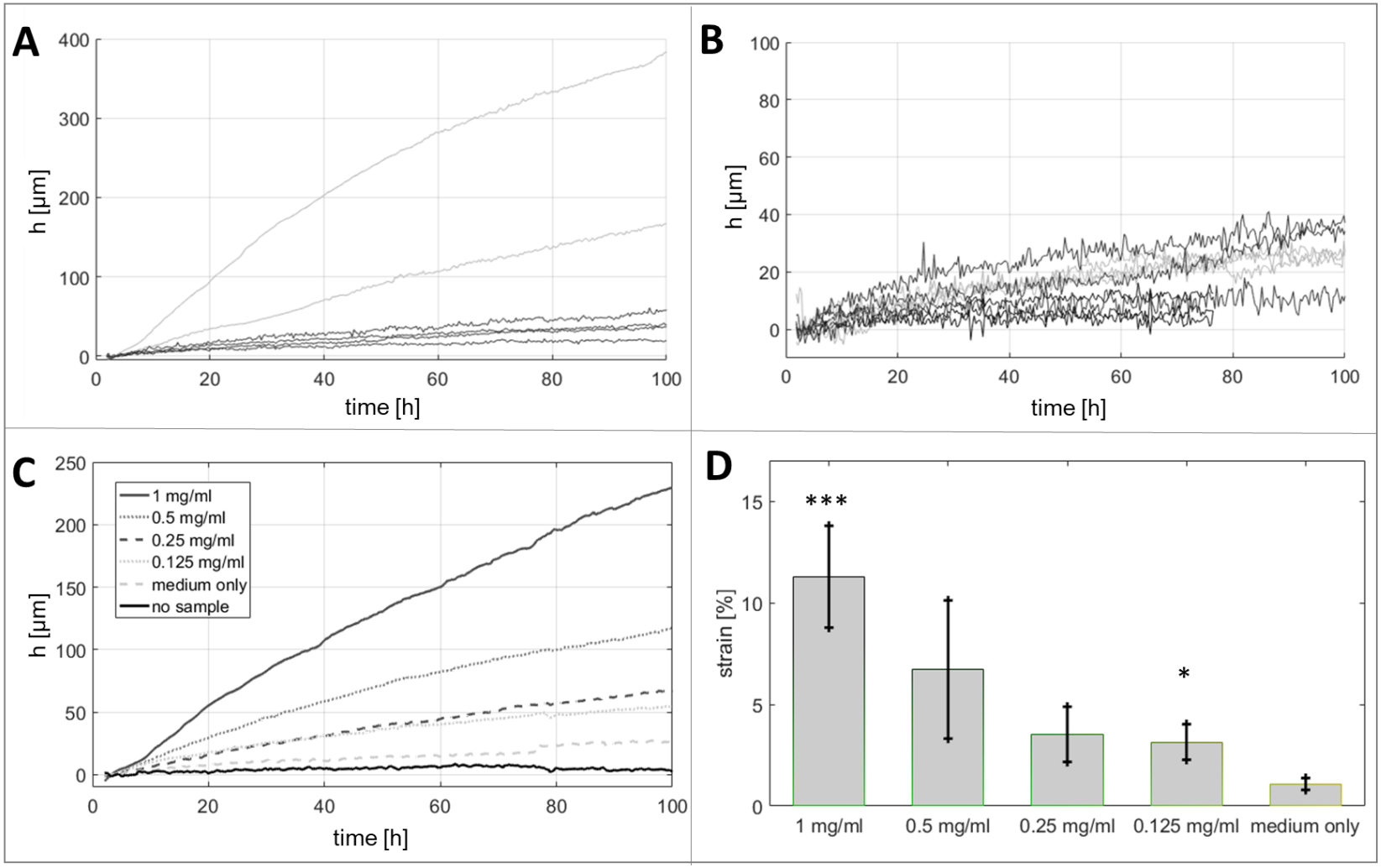


In-vitro system for real-time monitoring of cartilage degeneration



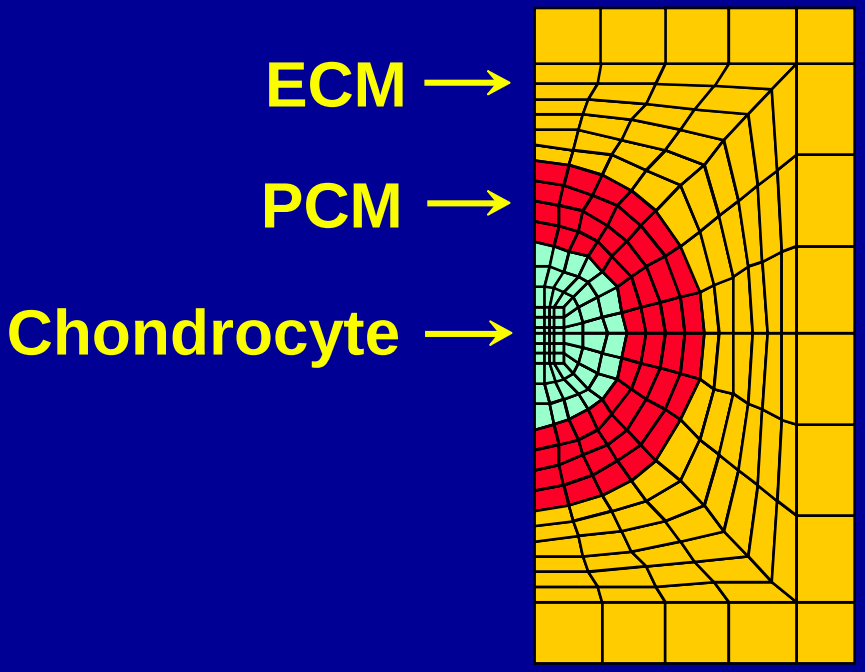

A
m
g
h
k1
degradation
k2
Normal sample
Degraded sample
$k_2 < k_1$
B
h_1
h_2
Normal sample
Degraded sample
$h_2 > h_1$
C
Height adjustment screw
Top plate
X-Y stage
Plate spacer
Distance sensor
Indenter
Sample housing
24-well plate
Sample
Kinematic coupling
Bottom plate
h
D
Top plate
Distance sensor
Indenter
Plate spacer
Kinematic coupling
Height adjustment screw
X-Y stage
Sample housing
Bottom plate
E
Buffer
Agarose gel
Sample
Indenter
Brass ring
Sample housing
24-well plate
Kinematic coupling
Bottom plate
F

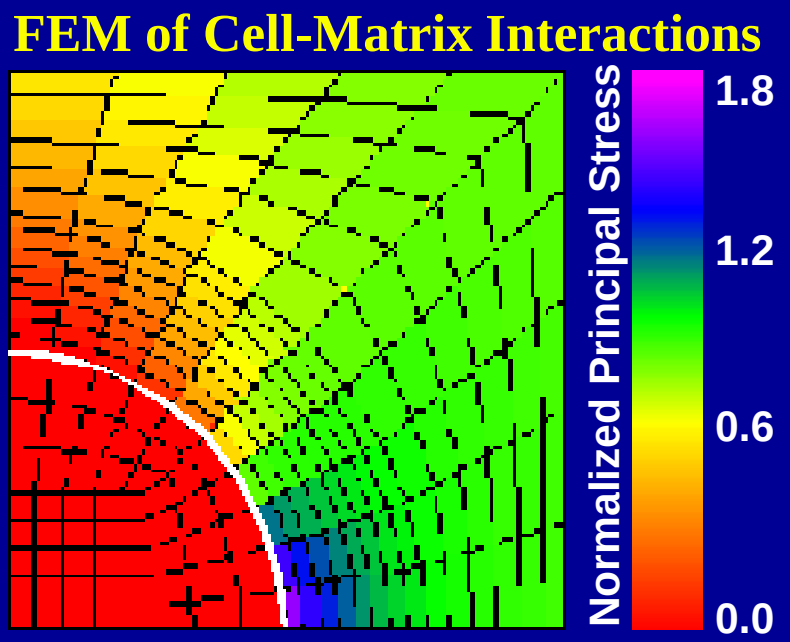

A
1) Place samples (on agarose pockets in a 24-well plate) in the housing
2) Place posts on samples using housing guides
3) Position housing on bottom plate using kinematic coupling
Takes place in a sterile laminar hood
Takes place in a sterile incubator
B
time
Thermal Stabilization
Sample preparation
Run experiment
•Place device in incubator
•24 h
•Assemble samples & indenters
•15 min
•Measure indenter displacement
•At least 72 h
C
1 2 3 4 5 6
A
B
C
D
D
Ø8mm
Ø6mm
1 2 3 4 5 6 7 8


A
h [µm]
time [h]
B
h [µm]
time [h]
C
1 mg/ml
0.5 mg/ml
0.25 mg/ml
0.125 mg/ml
medium only
no sample
h [µm]
time [h]
D
strain [%]
***
*
1 mg/ml
0.5 mg/ml
0.25 mg/ml
0.125 mg/ml
medium only

Mechanical environment of chondrocytes is important in cartilage homeostasis

What is the mechanical environment of chondrocytes and how it is altered with osteoarthritis?



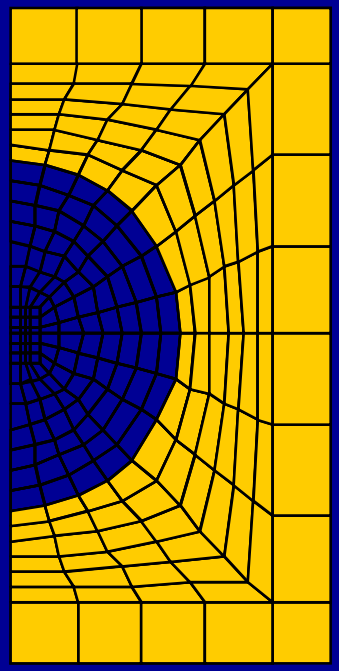


Mow et al., 1980; Mak et al., 1987; Frank & Grodzinsky, 1990; Soltz & Ateshian, 2000; Almeida et al., 1997; Guilak and Mow, 2000

Mechanical environment of chondrocytes is important in cartilage homeostasis

MECHANICAL PROPERTIES Extracellular Matrix (ECM)

ECM →



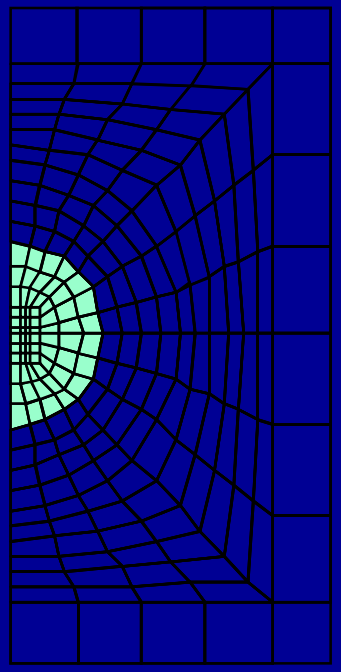
Young's modulus E [KPa]	~70-2000
Poisson's Ratio ν	~ 0.04
Time constant τ [s]	~ 5000

Athanasiou et al. (1991), Zhu et al. (1993), Chen et al. (2001), Hori et al. (1976), Schinagl et al. (1997), Mow et al. (1980)

Mechanical environment of chondrocytes is important in cartilage homeostasis

MECHANICAL PROPERTIES
Chondrocyte

Chondrocyte →



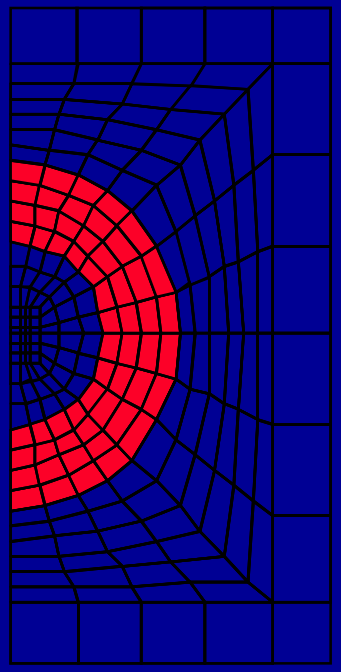
Young's modulus E [KPa]	~ 0.5
Poisson's Ratio ν	~ 0.4
Time constant τ [s]	~ 33

Knight et al. (2002), Trickey et al. (2000), Baaijens et al. (2004), Koay et al. (2003), Freeman et al. (1994), Guilak et al. (2002)

Mechanical environment of chondrocytes is important in cartilage homeostasis

MECHANICAL PROPERTIES
Pericellular Matrix (PCM)

PCM →



Young's modulus E [KPa]	~ ?
Poisson's Ratio ν	~ ?
Time constant τ [s]	~ ?

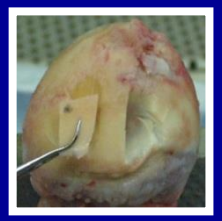
HYPOTHESES

1. **The mechanical properties of the PCM are altered with OA**

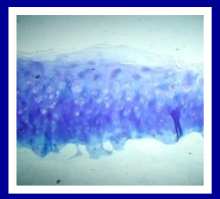
2. **The PCM regulates the mechanical environment of chondrocytes in normal and OA cartilage**

3. **Collagen type VI affects PCM stiffness, cartilage development, and progression of osteoarthritis**

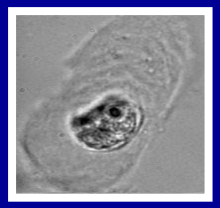
Material and Methods



- **Cartilage was obtained from human femoral heads (13 donors, age 19-75)**

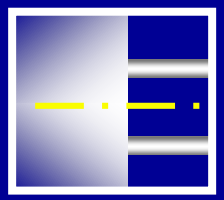


- **Cartilage (and chondrons) were classified as osteoarthritic (‘OA’) or non-osteoarthritic**

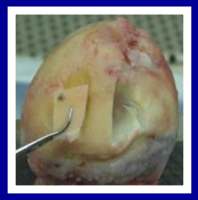


- **Chondrons were extracted from the surface and middle/deep zone (73 chondrons)**

- **The micropipette aspiration technique was performed in individual chondrons**

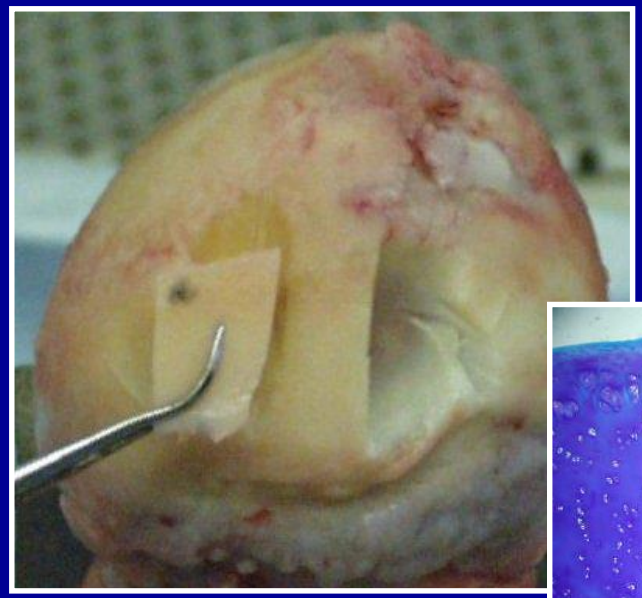


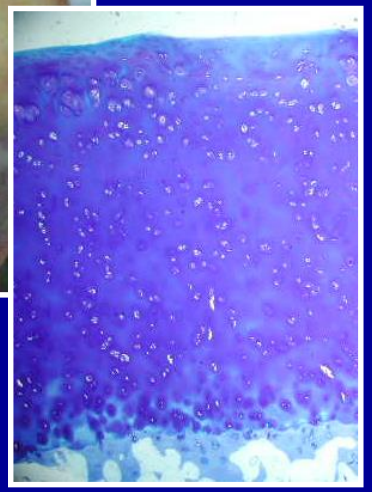
- **The mechanical properties of chondrons were measured using analytical and computational models**



Tissue harvesting / chondron grading

Non-OA Cartilage

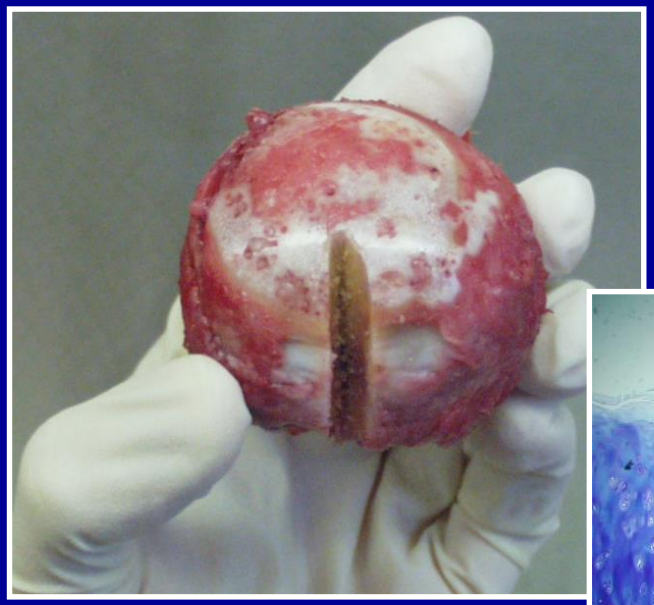


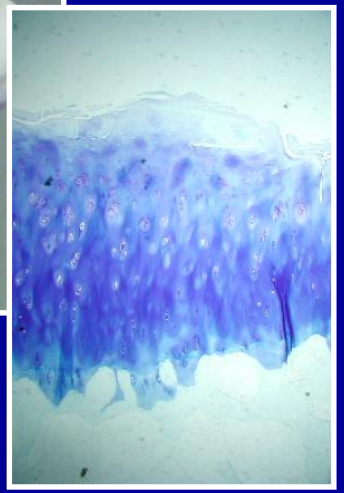


Average Grade

4.5

OA Cartilage

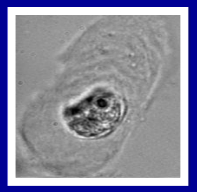




Average Grade

16

Carlson et al., 2002, Fink et al. 2002



Mechanical Chondron Isolation

Previously developed techniques:

Mechanical Isolation

[Poole et al., 1988]

Chondrons are isolated using a series of low speed homogenizations

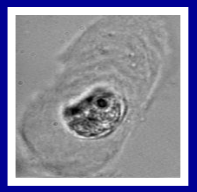
- ✓ **Retains PCM stiffness**
- × **Yields to small amount of chondrons (0~5)**
- × **Time consuming**
- × **Large amount of debris**

Enzymatic Isolation

[Lee et al., 1997]

Chondrons are isolated by digesting the cartilage with a mixture of dispase and collagenase

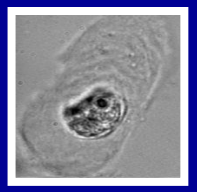
- ✓ **High yield of chondrons**
- ✓ **Small amount of debris**
- × **Compromises the PCM stiffness**



Mechanical Chondron Isolation

MICROASPIRATOR:

Alexopoulos et al. 2003

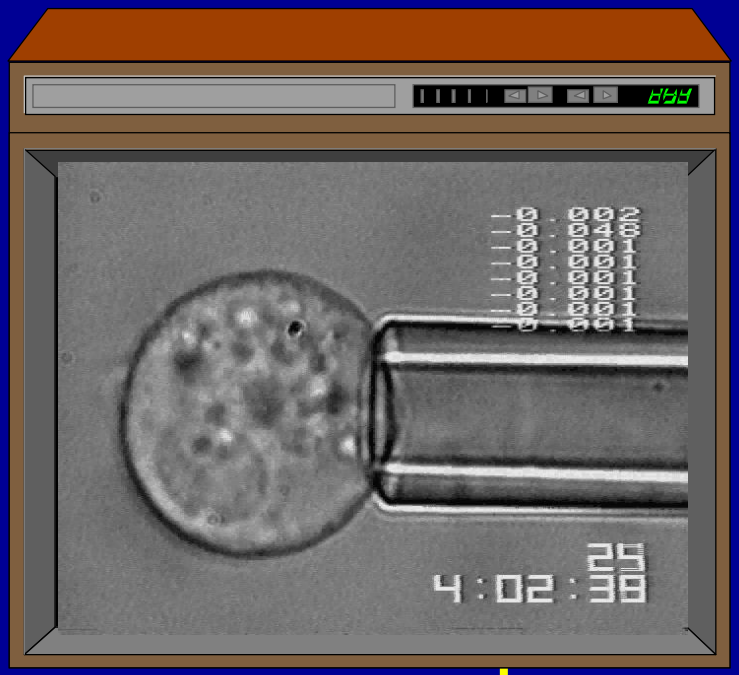


Mechanical Chondron Isolation

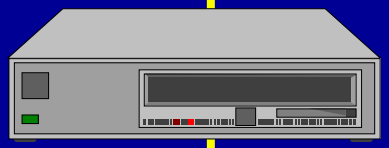
MICROASPIRATOR

- ✓ Retains PCM stiffness
- ✓ Small amount of debris
- ✓ It takes 10 minutes
- ✓ Site specific
- ✓ Requires very small amount of cartilage (area)
- ✓ Can be applied in murine cartilage
- ✗ Moderate yield of chondrons (ten to hundreds)

Micropipette aspiration technique

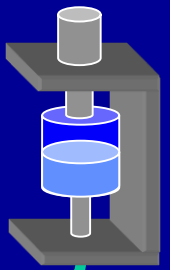


Monitor



VCR

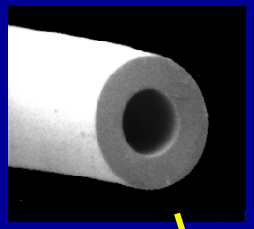
Multiplexer



Fluid reservoir

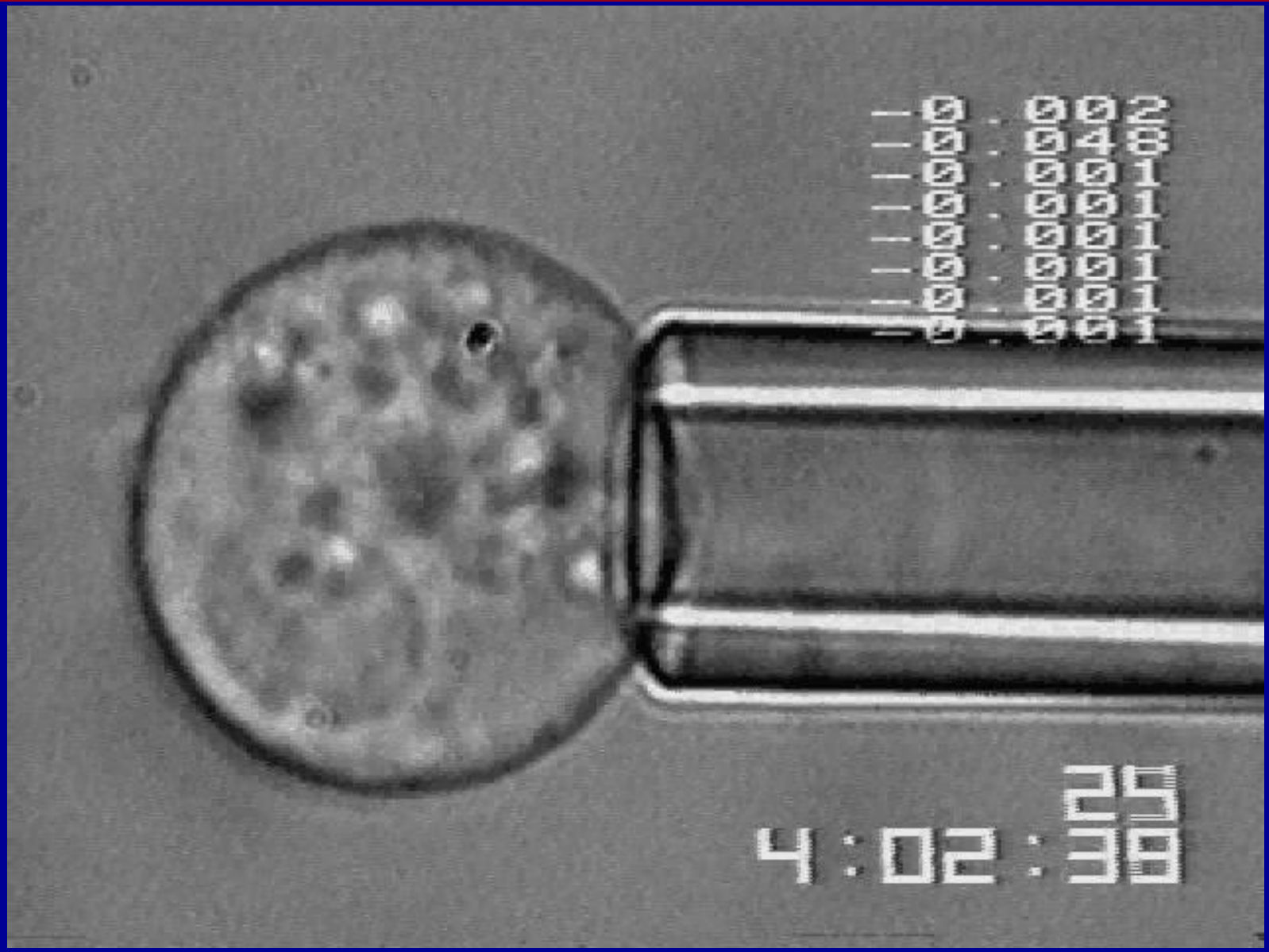
Micromanipulator

Micropipette



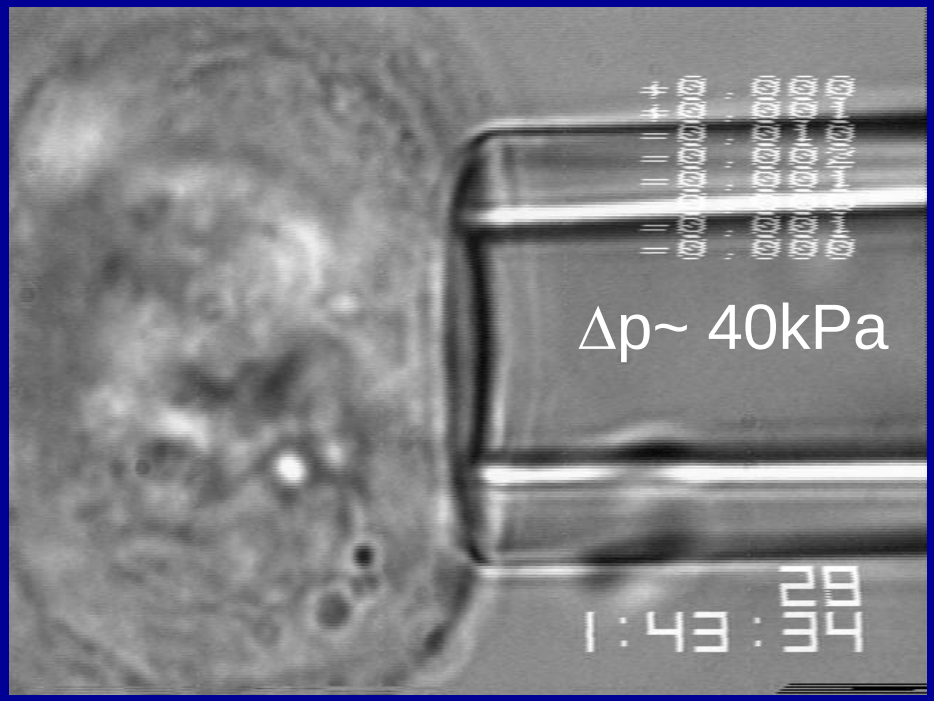
Pressure transducer

Micropipette aspiration technique



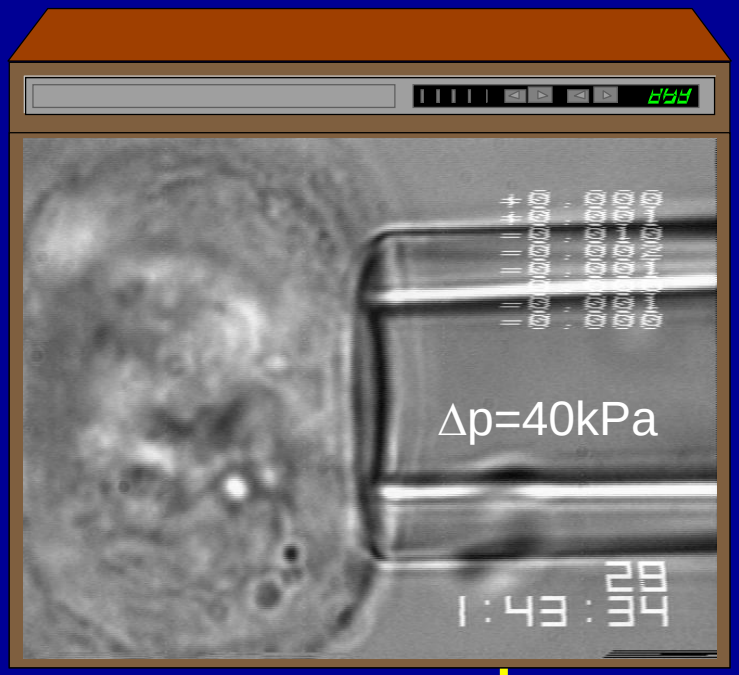
Young's modulus for different chondron isolation techniques

Mechanical Isolation



Enzymatic Isolation

Micropipette aspiration technique



Monitor

VCR

Multiplexer

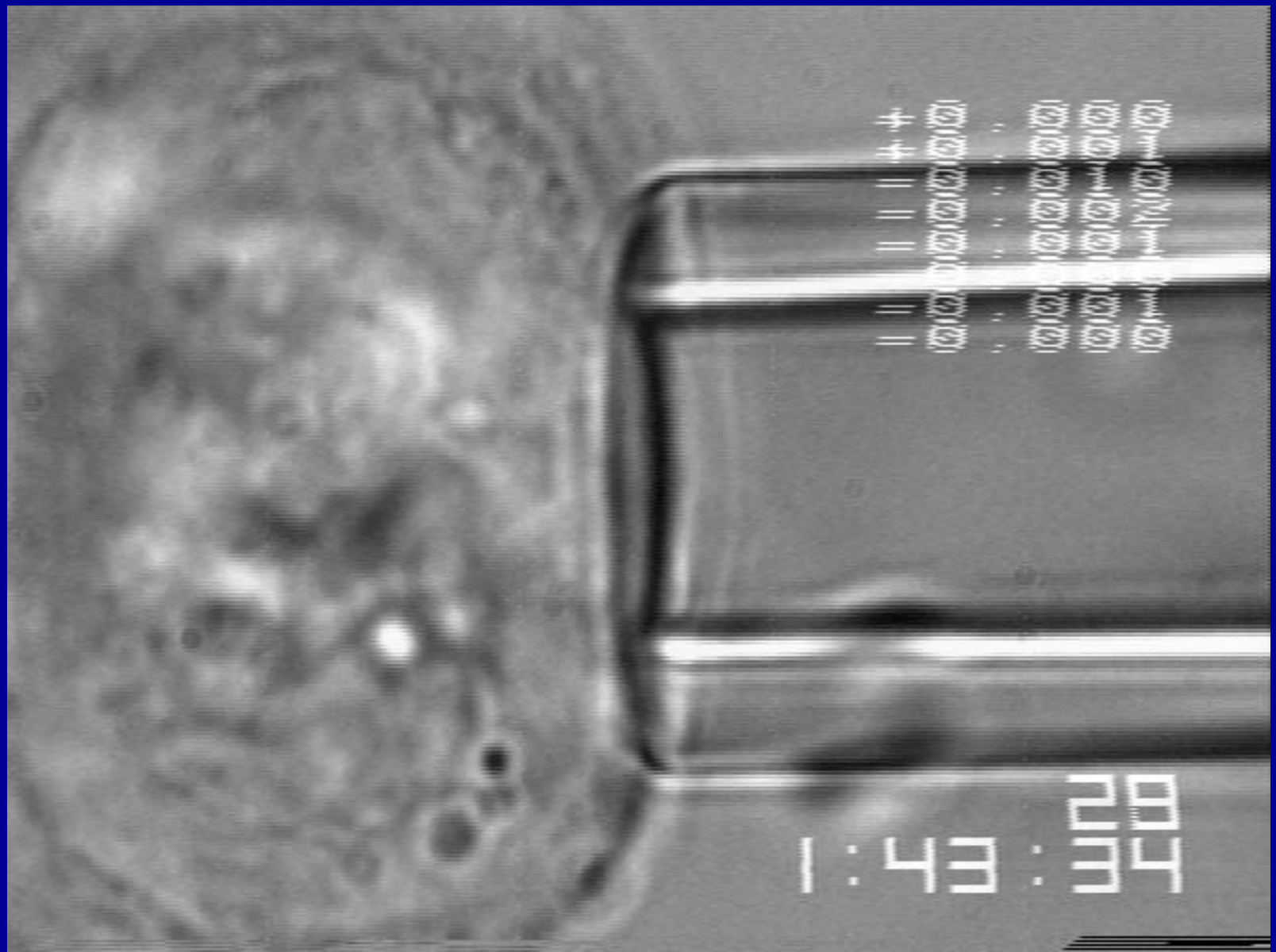
Micropipette

Micromanipulator

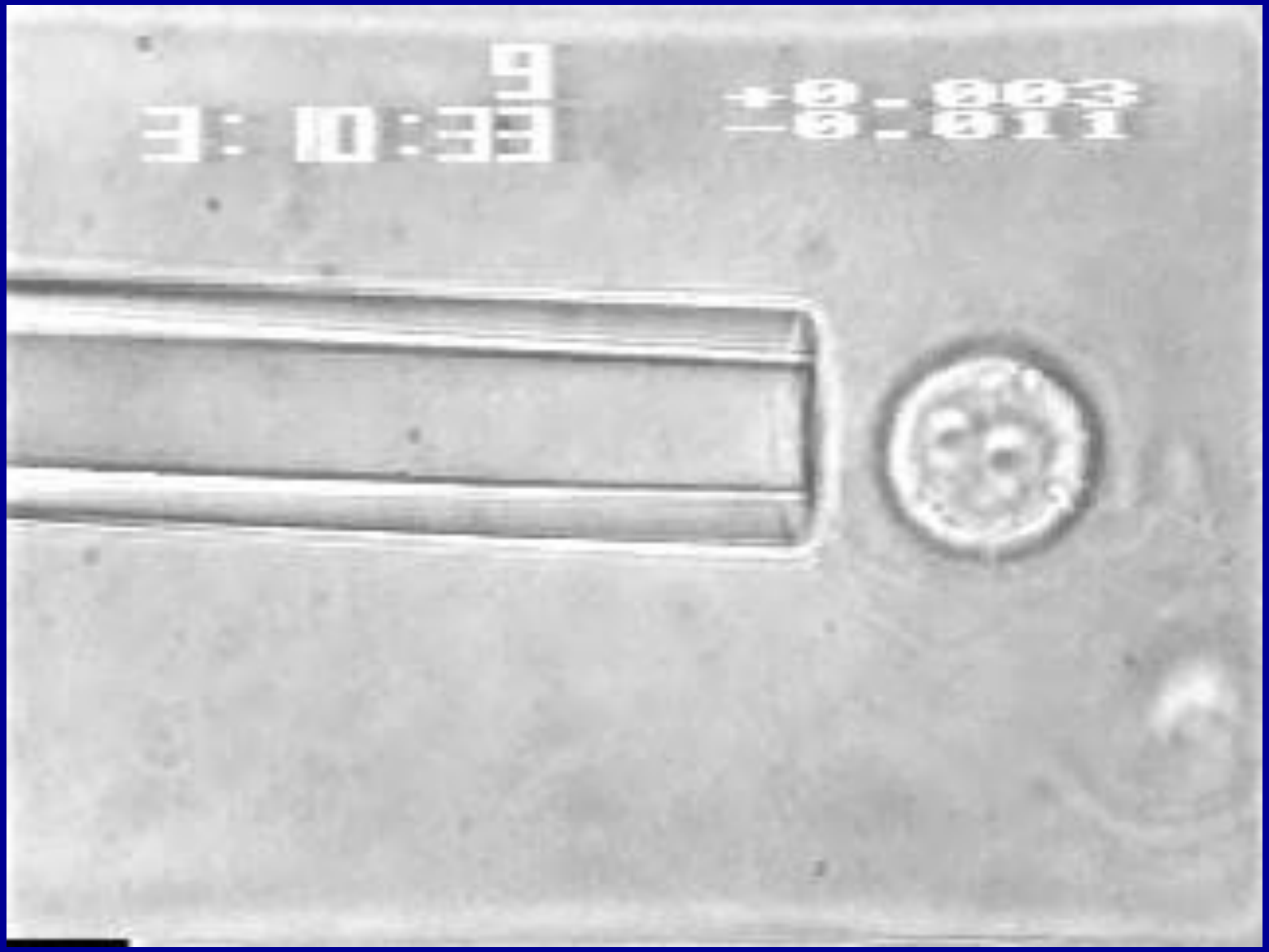
Syringe

Pressure transducer

Micropipette aspiration technique



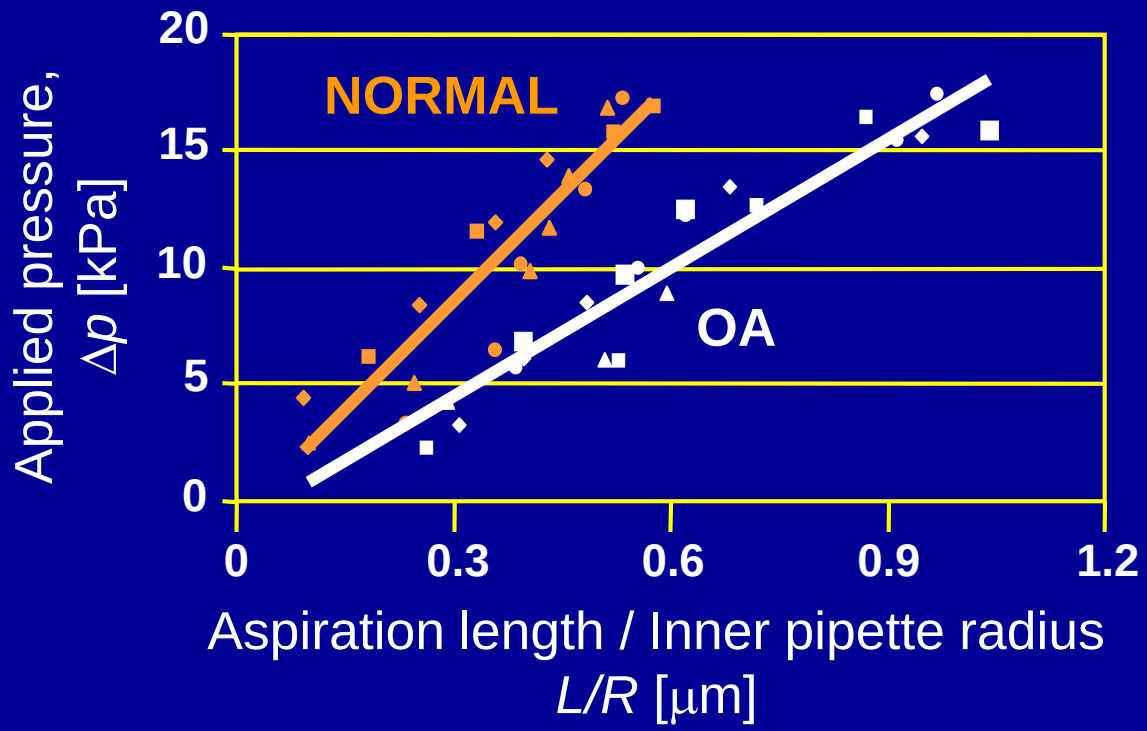
Micropipette aspiration technique



How to measure the mechanical properties

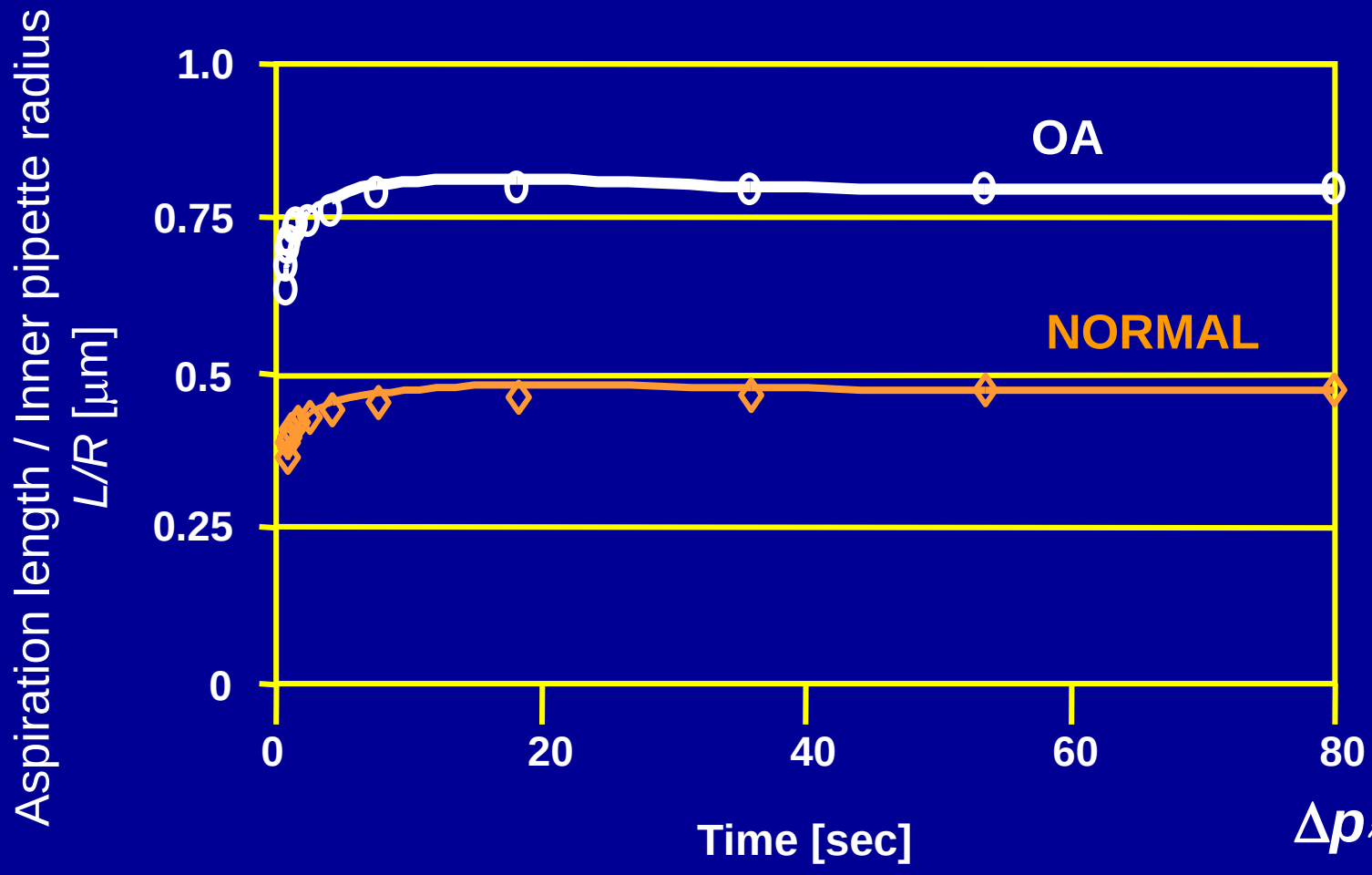
EQUILIBRIUM TESTS were used to quantify the Young's modulus, E, of the PCM:

$$E \sim \left(\frac{\Delta p}{L / R} \right)$$



How to measure the mechanical properties

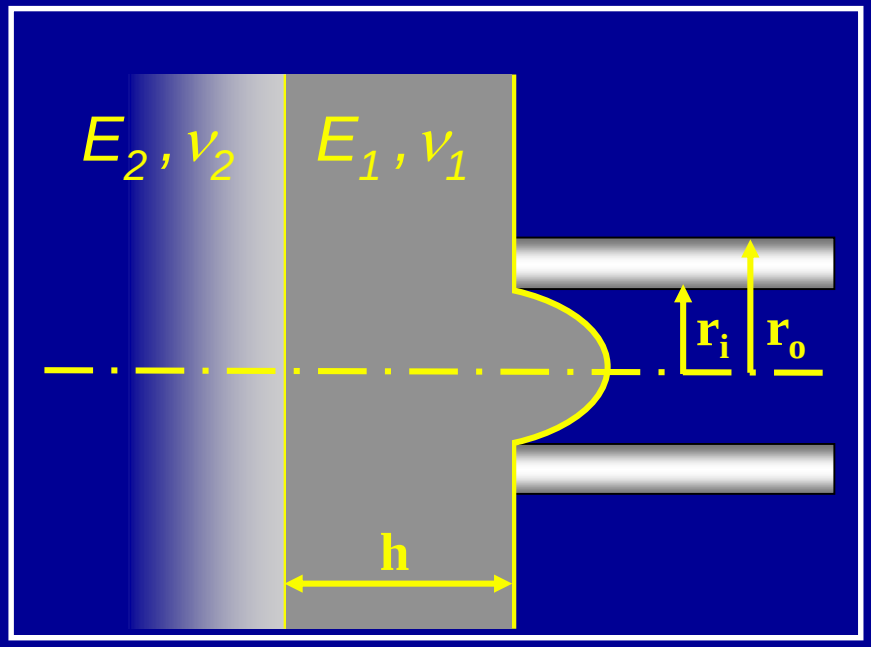
CREEP TESTS were used to quantify the Young's modulus, permeability and Poisson's ratio of the PCM:

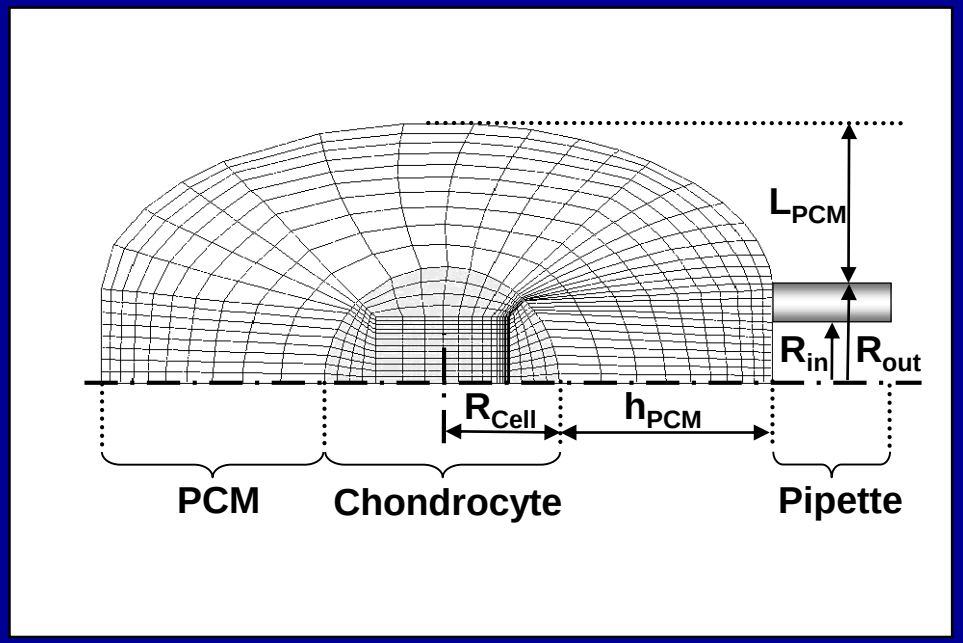


Mathematical Models for Chondrons

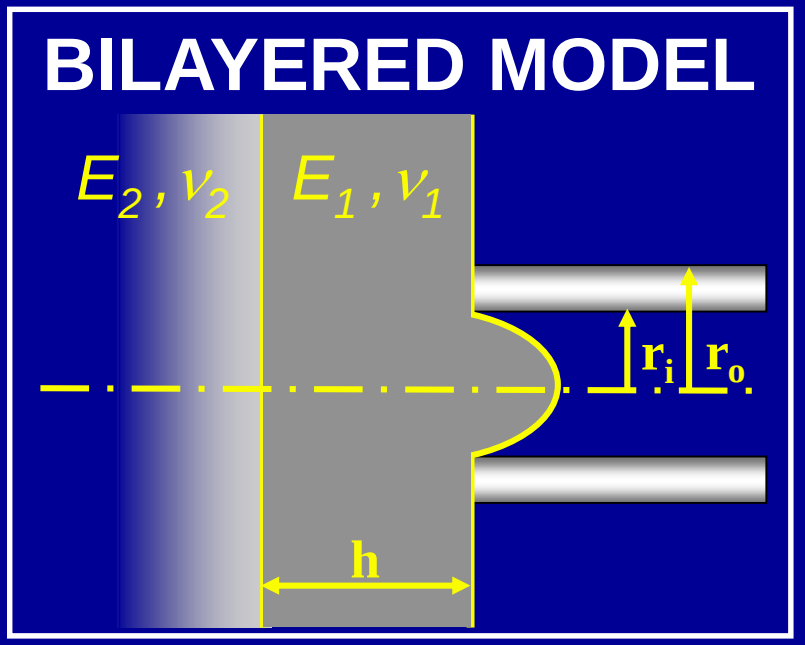
Analytical Model

Computational Model





Analytical Model for Chondrons



ASSUMPTIONS

- Homogeneous
- Isotropic
- Elastic, Small strains
- Half space substrate
- Solid phase only

ADVANTAGES

- Bilayer (cell+PCM)
- Analytical (exact solutions)

Young's Modulus, E_1

Formulation: Governing Equations

The governing equations of equilibrium for an isotropic, compressible elastic solid are:

Equation of equilibrium: $div\mathbf{S} = 0$

Small strain equations: $\mathbf{E} = \frac{1}{2}\left[\nabla \mathbf{u} + \nabla \mathbf{u}^{T} \right]$

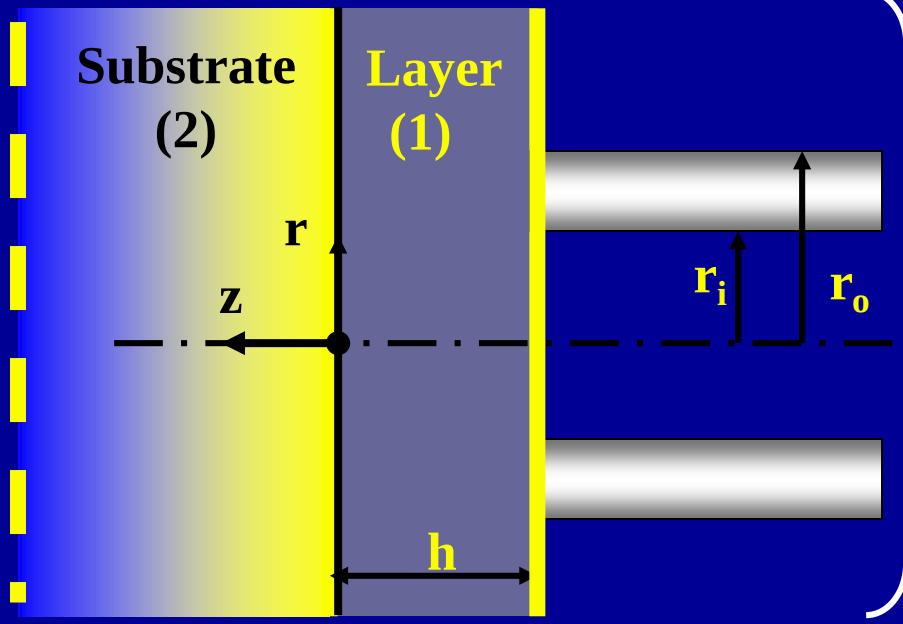
Constitutive equations: $\mathbf{S} = \lambda tr(\mathbf{E})\mathbf{I} + 2\mu\mathbf{E}$

Navier's equation of equilibrium: $\nabla div\mathbf{u} + (1-2\nu)\nabla^2\mathbf{u} = 0$

Papkovich-Neuber solution has been used to solve the Navier's equations

Formulation: Boundary Conditions

At $z \to \infty$.
All stresses
equal to 0.



In the surface (z=-h):

$\sigma_z^{(1)}(r,-h)=0$

$u_z^{(1)}(r,-h)=0$

$\sigma_z^{(1)}(r,-h)=\Delta p$

$\sigma_{rz}^{(1)}(r,-h)=0$

At z=0 (continuity conditions):

Stresses$^{(1)}$ = Stresses$^{(2)}$

Displacements$^{(1)}$ = Displacements$^{(2)}$

Theoretical solution

The displacement $\vec{u}$ was expressed using the Papkovich-Neuber solution:

$$2\mu\vec{u} = -4(1-\nu)\ \vec{\psi} + \nabla(\vec{r}\cdot\vec{\psi}+\varphi)$$

where: r is the position vector

$\vec{\psi}$ is the Papkovich-Neuber vector and

φ is the harmonic strain potential

- These displacements equations readily satisfy the governing equations if $\vec{\psi}$ and φ are harmonic functions.

- A common way to express the harmonic functions is the Hankel transform method developed by Sneddon (1947).

Theoretical solution : Hankel Transform

The stress profile in the surface takes the form:

$$\sigma_{zz}^{(1)}\Big|_{\text{surface}} = \int_0^{\infty} s \cdot N(s) \cdot J_0(sr) \cdot ds$$

Where the unknown function *N(s)* corresponds to the Hankel transform of the normal stress in the surface:

The normal stresses

$$\sigma_{zz}^{(1)}\Big|_{\text{surface}} = \begin{cases} \Delta p & , \ 0 \le r \le r_i \\ \dfrac{-2\Delta p \cdot r_i \cdot f(r)}{\pi \cdot \sqrt{(r_0^2 - r^2)\cdot(r^2 - r_i^2)}} & , r_i \le r \le r_0 \\ 0 & , \ r_0 \le r \end{cases}$$

The corresponding Hankel transform

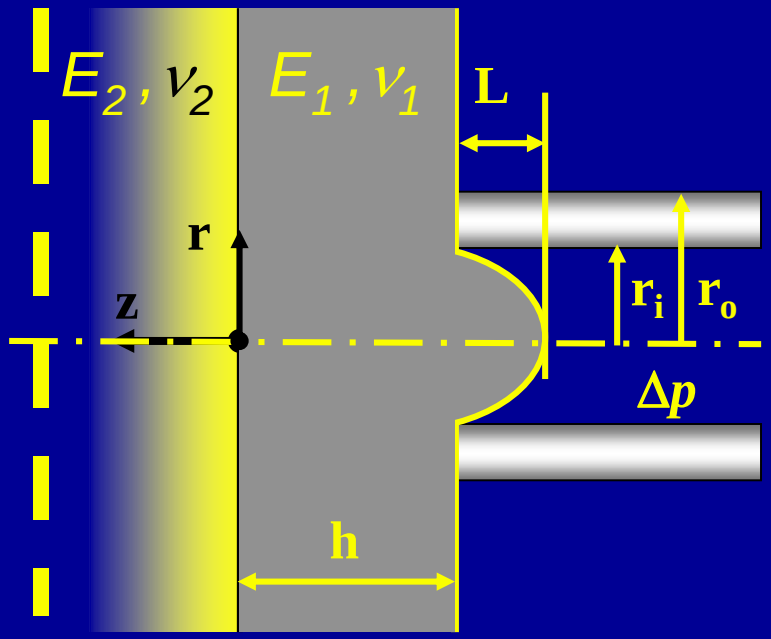
$$\begin{cases} \Delta P \cdot r_i \cdot \dfrac{J_1(sr_i)}{s} \\ -\Delta P \cdot r_i \cdot \sum_{n=0}^{\infty} \alpha_n \cdot J_n(sr_c) \cdot J_n(sb) \\ 0 \end{cases}$$

Theoretical solution: Aspiration length

$$E_1 = C \cdot \left(\frac{\Delta p}{L / r_i} \right)$$

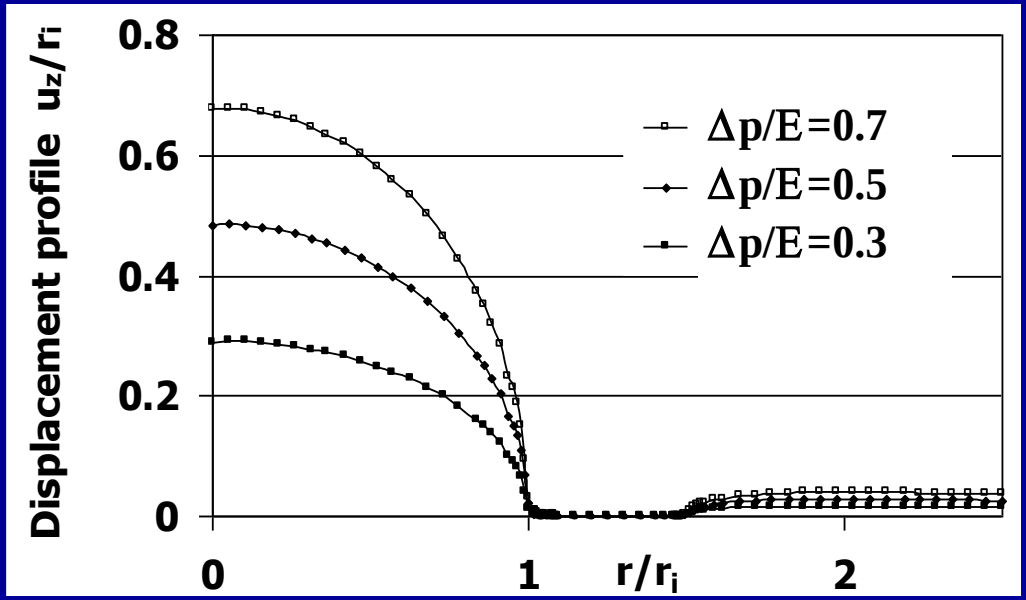
C is a function of 5 parameters:

- ✓ the Young's moduli ratio E_1/E_2
- ✓ the Poisson's ratio ν_1
- ✓ the Poisson's ratio ν_2
- ✓ the thickness of the layer $h^*=h/r_i$
- ✓ the thickness of the pipette wall $r_0^* = r_0 / r_i$

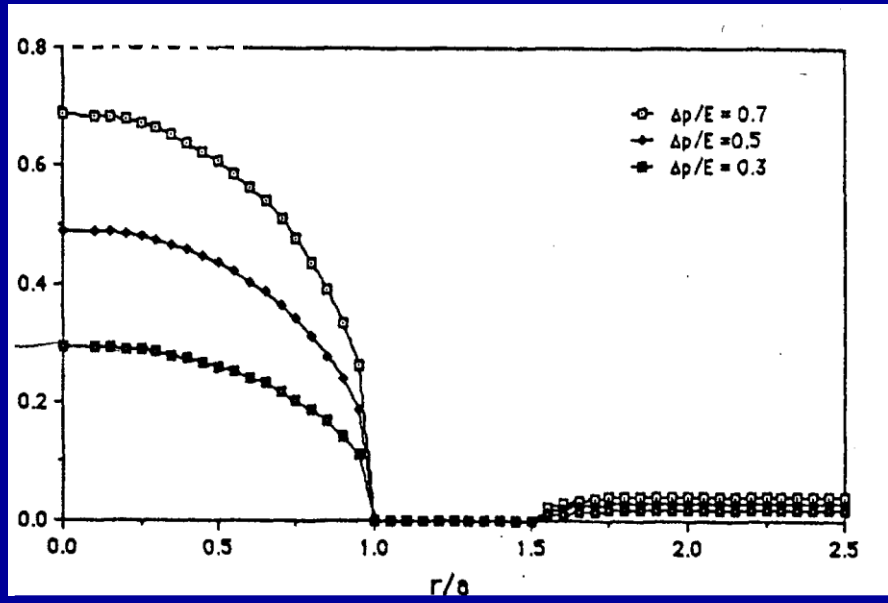


Validation using Theret's solution

Present solution

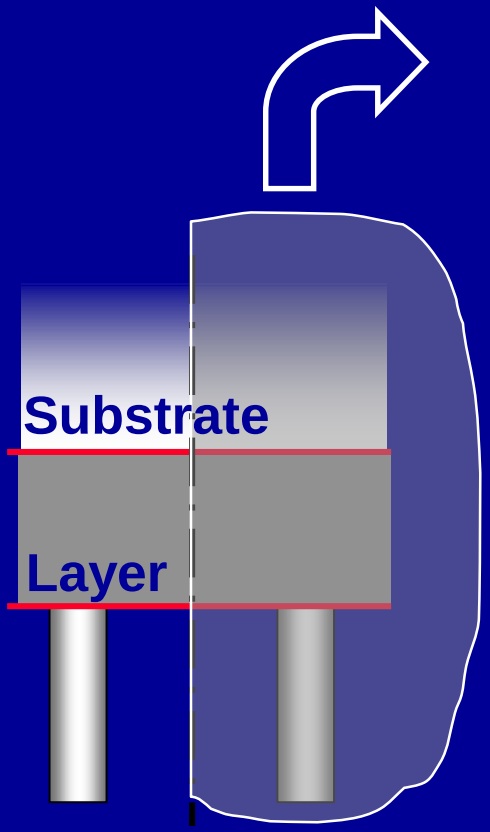


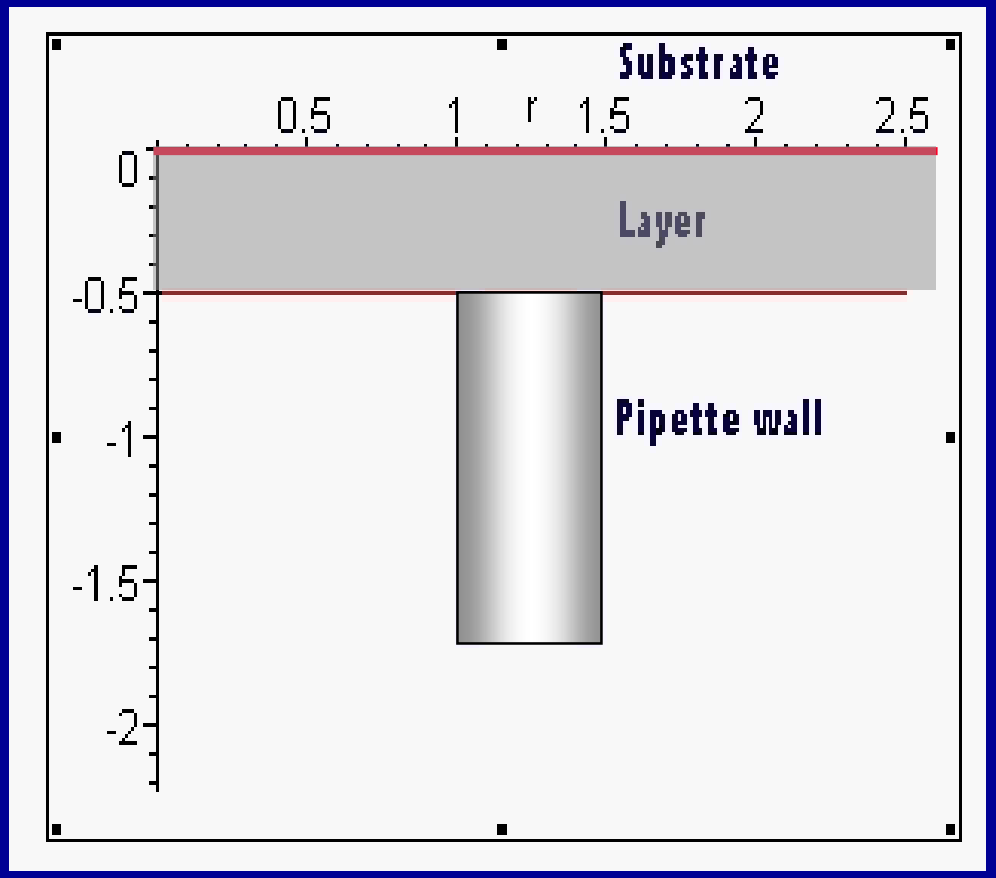
Theret's half-space



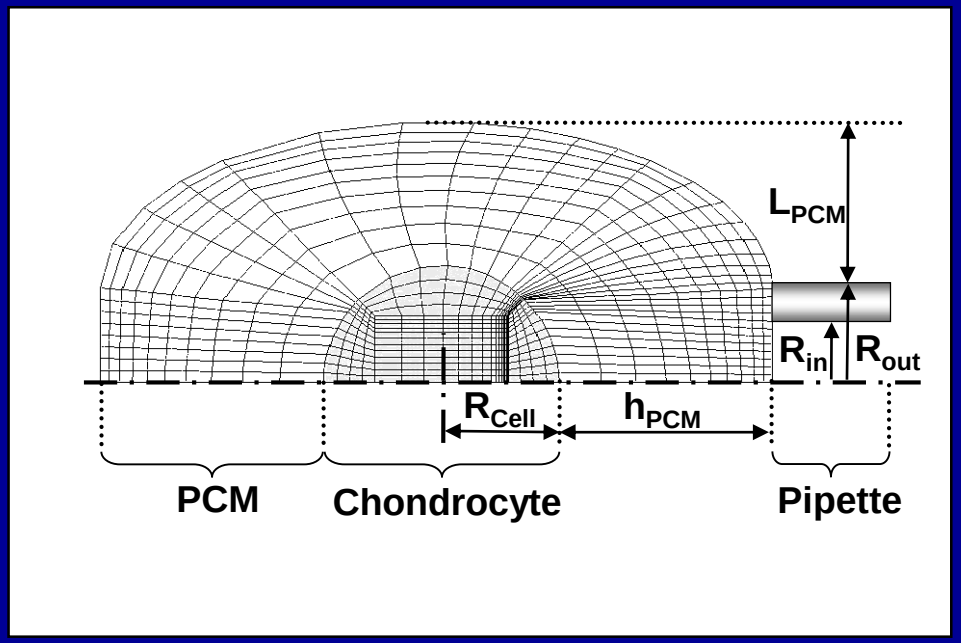
Theret et al. (1987) half-space model (right) is in very good agreement with the present solution (left).

Analytical Model for Chondrons





Computational Model for Chondrons



ADVANTAGES

- Biphasic
- Exact geometry

Young's Modulus, Permeability, Poisson's Ratio

Biphasic Formulation

Continuity $\nabla \cdot (\varphi^{f}\mathbf{v}^{f} + \varphi^{s}\mathbf{v}^{s}) = 0, \quad \varphi^{f} + \varphi^{s} = 1$

Balance of linear momentum $\nabla \cdot \boldsymbol{\sigma}^{s} + \boldsymbol{\pi}^{s} = 0, \quad \nabla \cdot \boldsymbol{\sigma}^{f} + \boldsymbol{\pi}^{f} = 0, \quad \boldsymbol{\pi}^{s} = -\boldsymbol{\pi}^{f}$

Constitutive equations

$$\boldsymbol{\sigma}^{f} = -\varphi^{f} p\mathbf{I}$$

$$\boldsymbol{\sigma}^{s} = -\varphi^{s} p\mathbf{I} + \boldsymbol{\sigma}^{e} \quad \boldsymbol{\sigma}^{e} = \lambda^{s}\mathrm{tr}(\mathbf{e}^{s})\mathbf{I} + 2\mu^{s}\mathbf{e}^{s}$$

$$\boldsymbol{\pi}^{s} = p\nabla\varphi^{s} - K(\mathbf{v}^{f} - \mathbf{v}^{s}) \quad K = \frac{\phi^{f\,2}}{k}$$

where:

ϕ	volume fraction (s: solid phase, f: fluid phase)
$\mathbf{v}^{f}$, $\mathbf{v}^{s}$	velocities of the fluid and solid phases
$\boldsymbol{\sigma}^{e}$	effective or elastic stress tensor
p	true fluid pressure
K	diffusive drag coefficient
k	permeability
$\boldsymbol{\pi}^{s \text{ or } f}$	momentum exchange between phases

Finite Element Formulation

The strong form of the governing equations is:

$$\nabla \cdot (\mathbf{v}^{s} - k\nabla p) = 0$$

$$\nabla \cdot (\boldsymbol{\sigma}^{e} - p\mathbf{I}) = 0$$

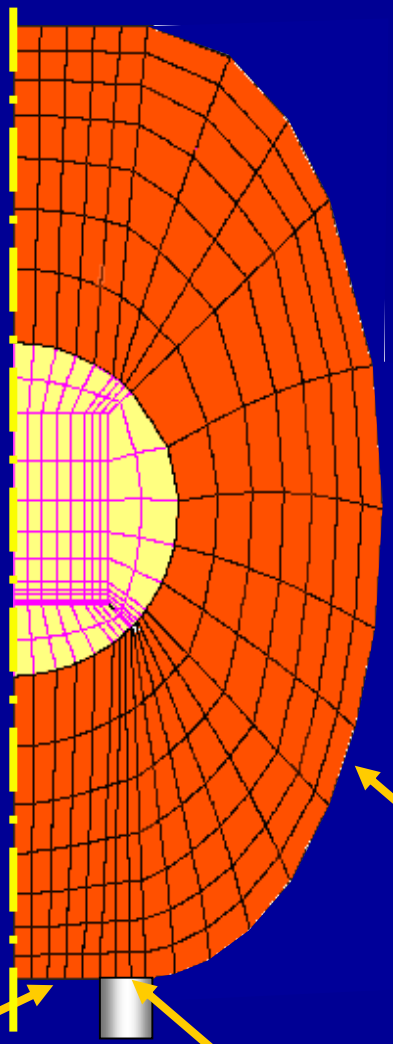
- **u**-*p* formulation (**u** and *p* are essential variables)
- Bilinear quadrilateral elements

ASSUMPTIONS

- Linear, Isotropic, Homogeneous solid matrix
- Inviscid fluid, intrinsically incompressible
- No inertia, No body forces
- Constant permeability

Boundary Conditions

Finite element mesh
(342 nodes, 314 elements)



$impermeable,\ u_r = 0$

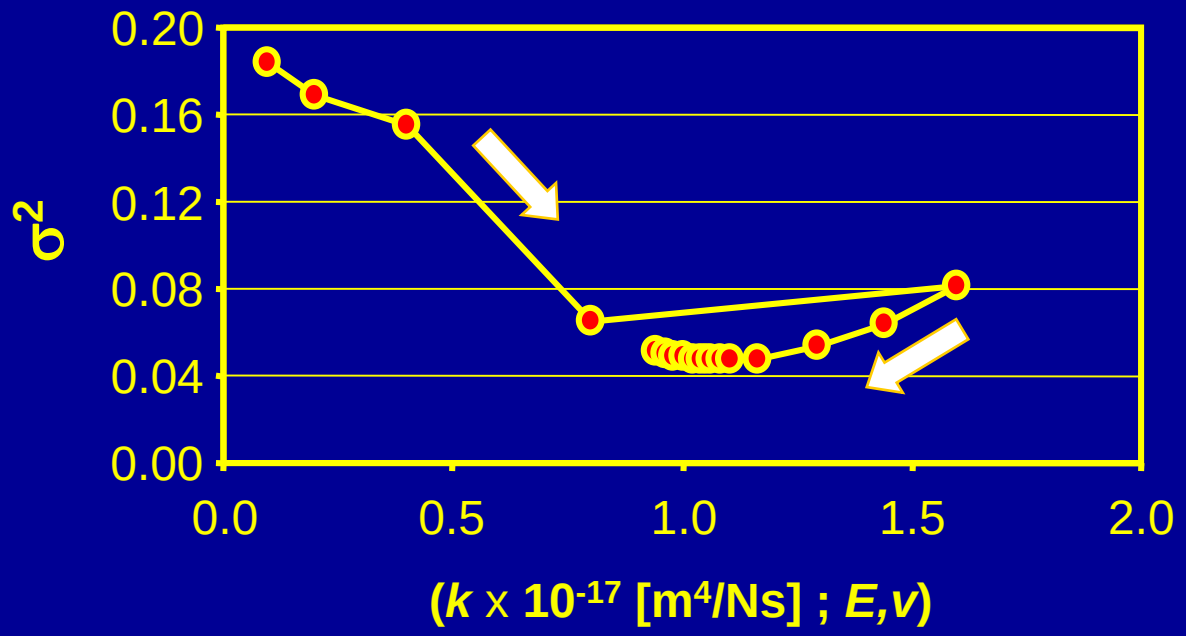
$p = 0$

$p = \Delta p,\ \sigma^e \vec{n} = 0$

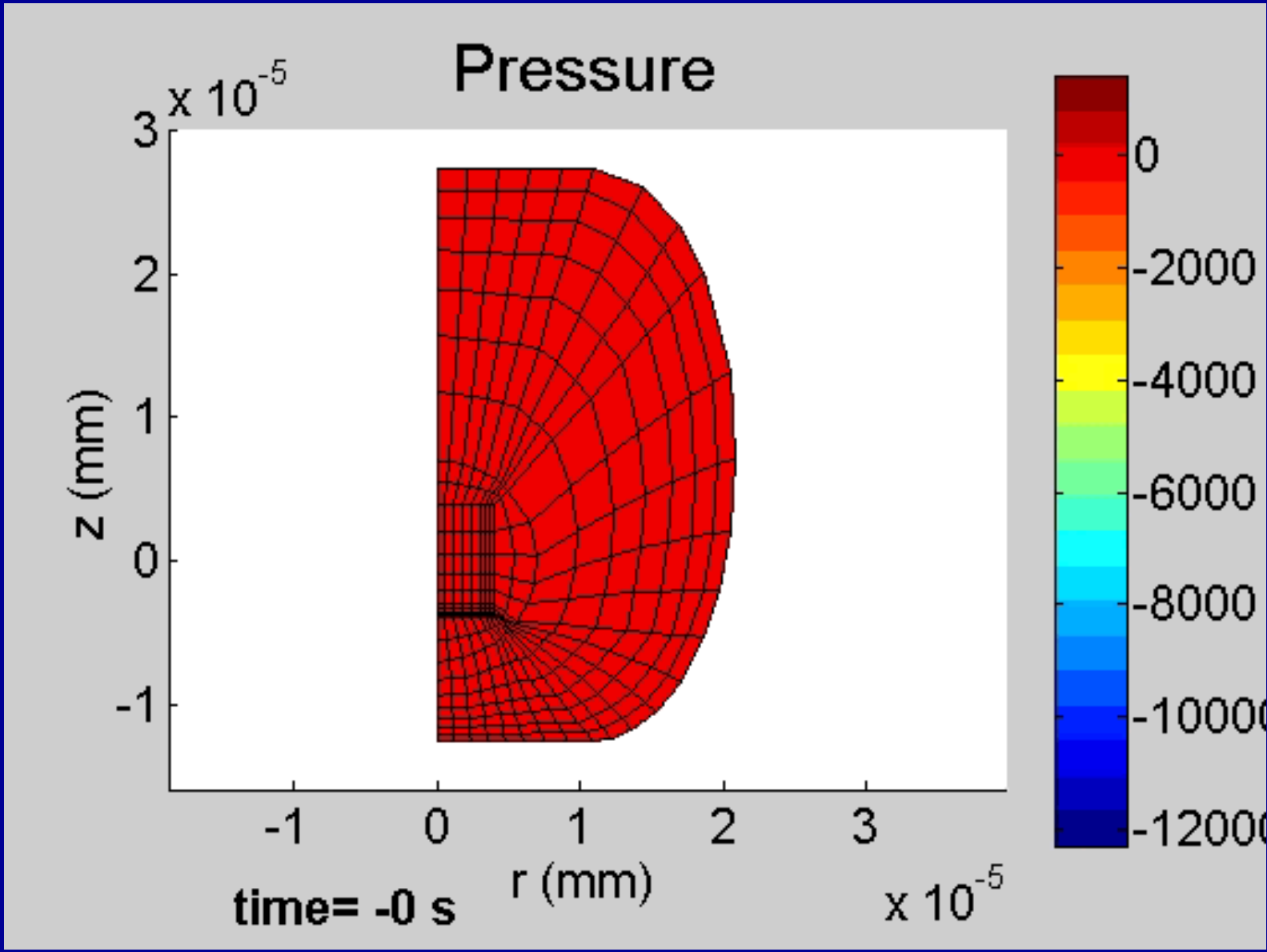
$u_z = 0,\ impermeable$

Optimization code

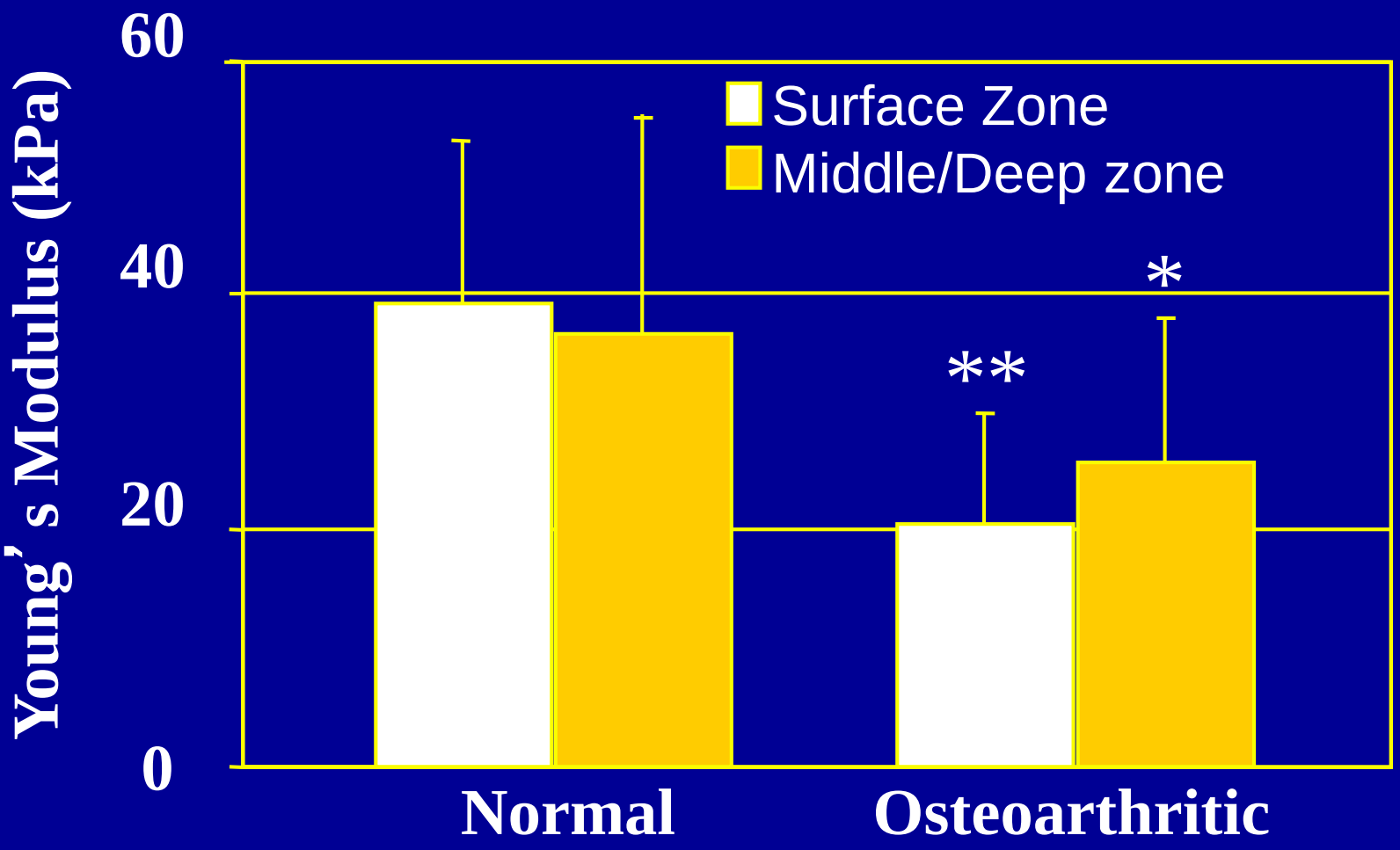
- Custom written optimization code
- Minimizes the sum of squared errors between the experimental data and the predicted aspiration length



Computational Model for Chondrons

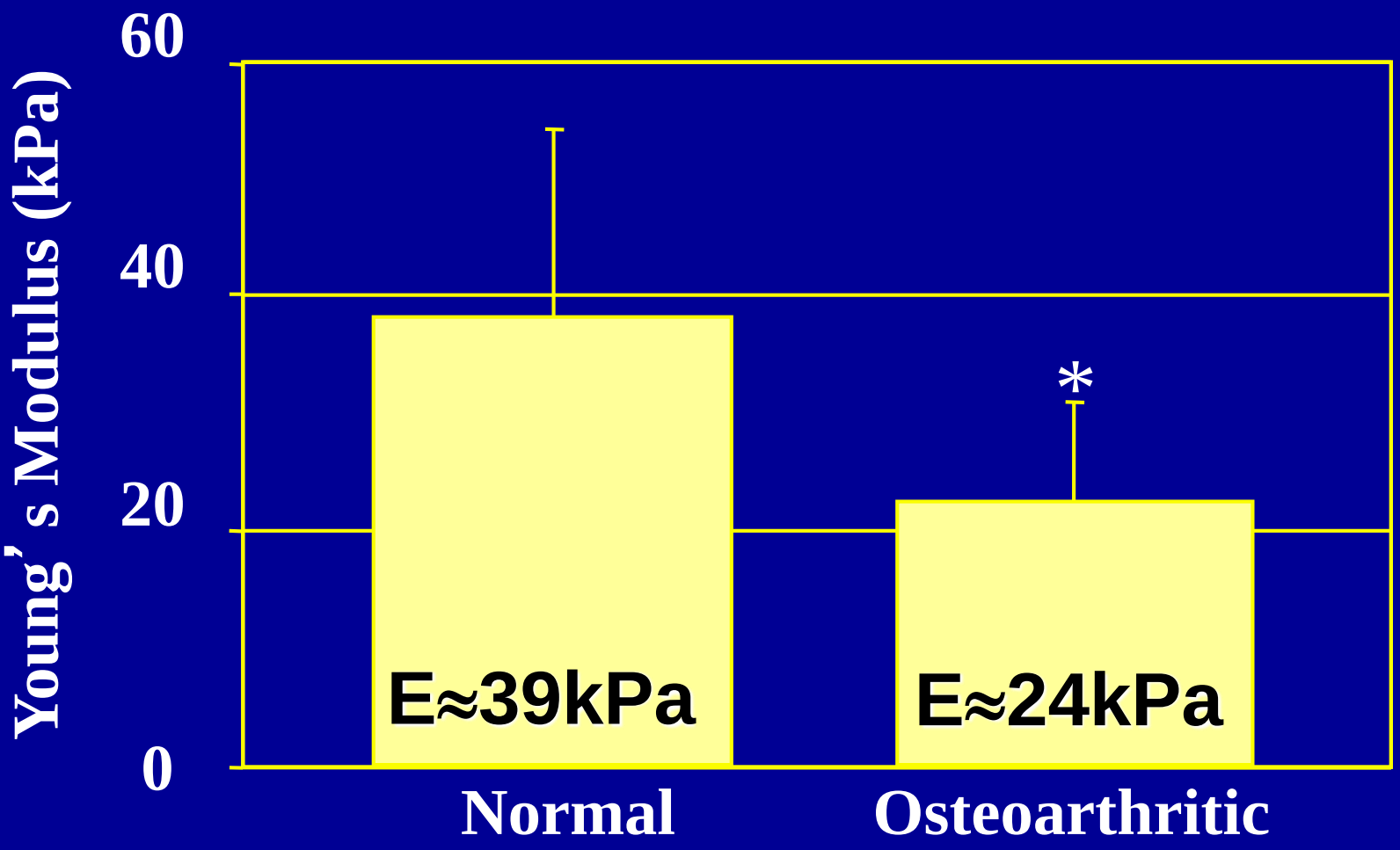


No zonal differences in the Young's modulus



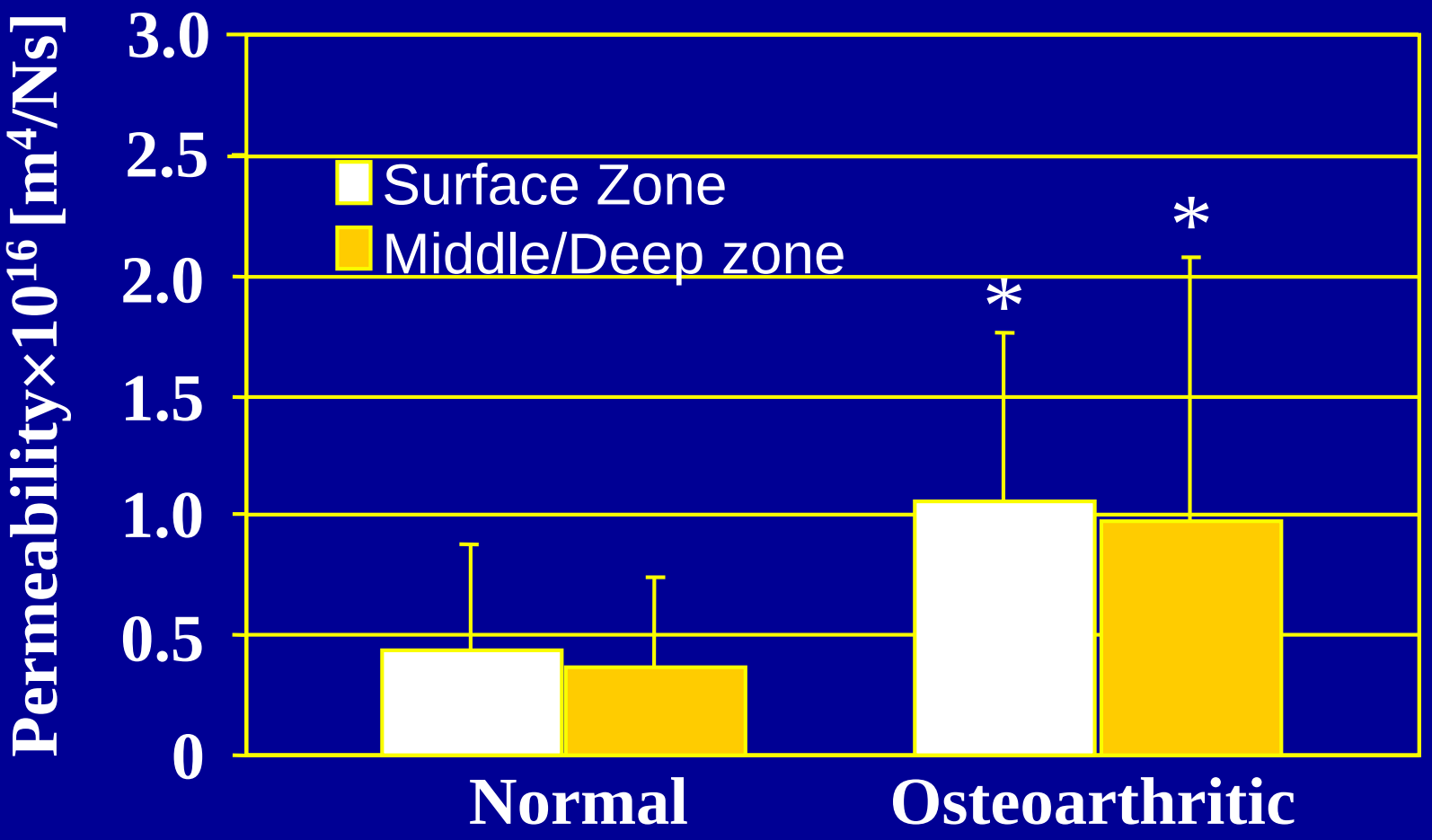
N>15 per group, **p< 0.0001, *p< 0.03 vs. the non-OA counterpart

Young's modulus is reduced with OA



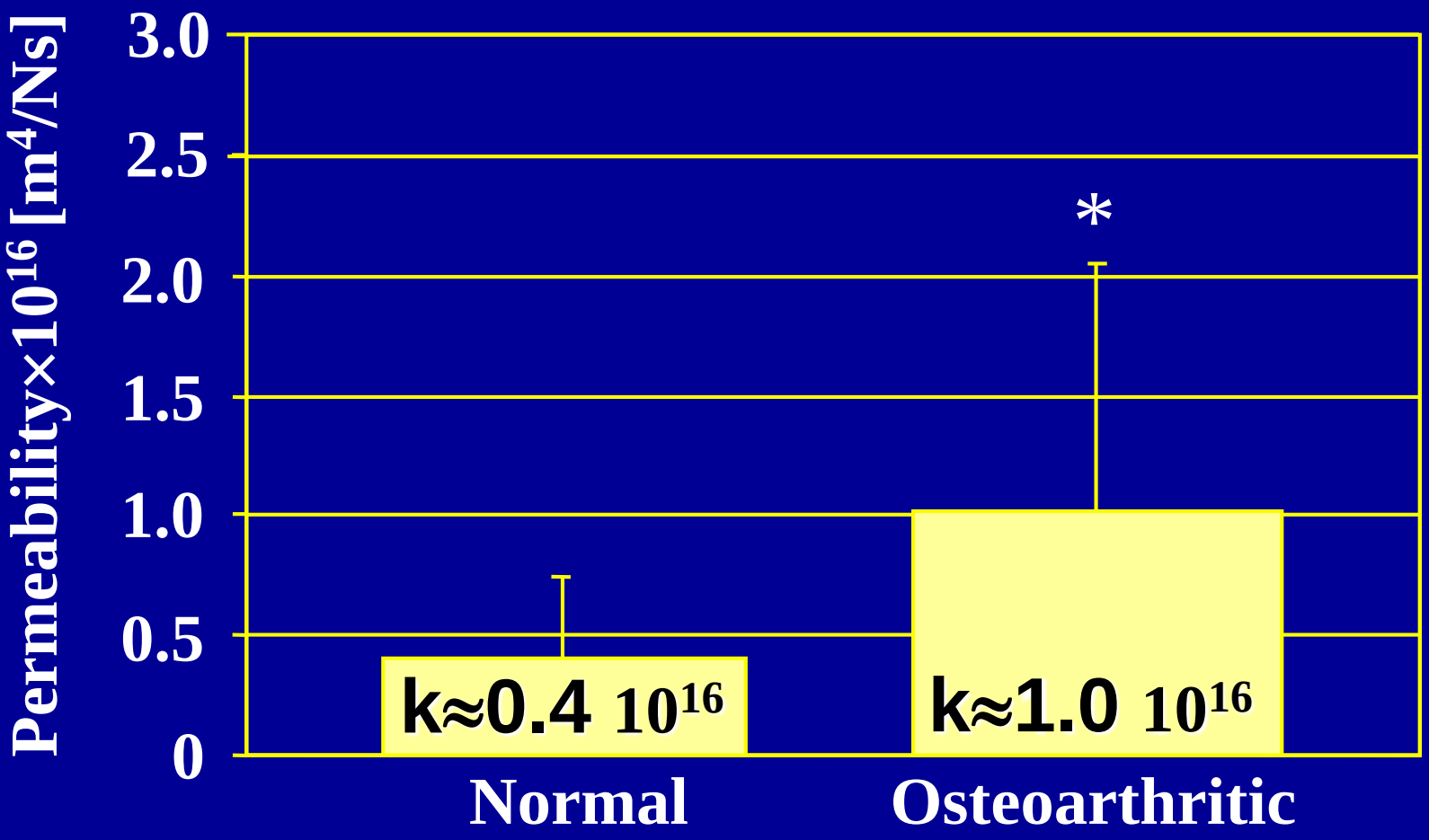
N>30 per group, *p<0.001 vs. the non-OA controls

No zonal differences in permeability



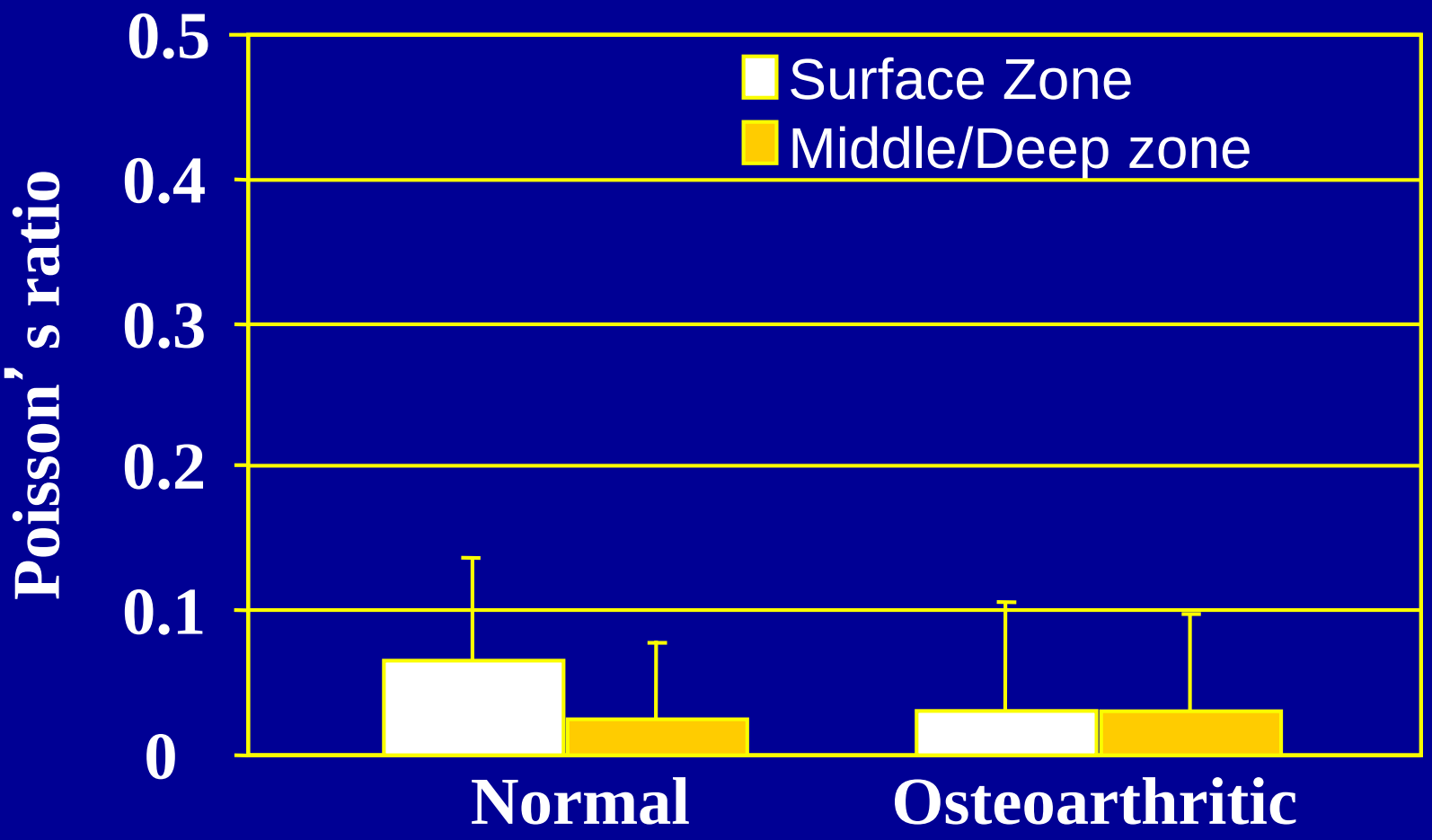
N>15 per group, *p< 0.03 vs. the non-OA counterpart

Permeability is increased with OA



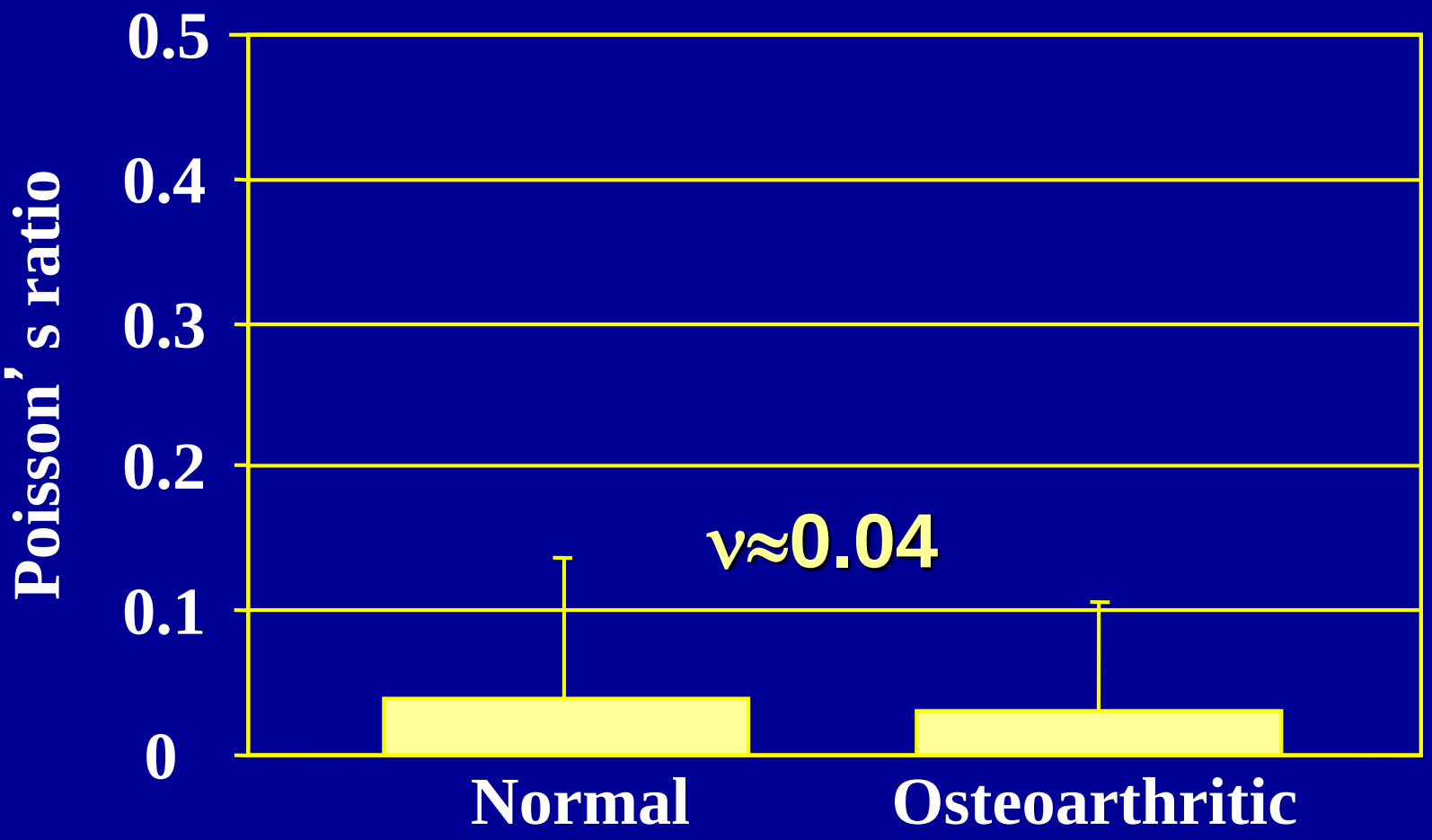
N>30 per group, *p< 0.0007 vs. the non-OA counterpart

No zonal differences in the Poisson's ratio



N>30 per group, p> 0.4, vs. the non-OA counterpart

No differences in the Poisson's ratio with OA



N>15 per group, p> 0.4, vs. the non-OA counterpart

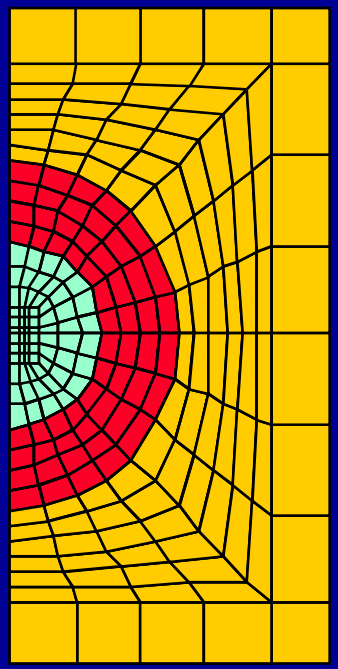
Conclusions: Young's Modulus

- PCM is softer than ECM
- PCM is ~100 times more stiff than the cell

ECM: 70-2000 kPa →

PCM: ~40 kPa →

Cell: ~0.5 kPa →

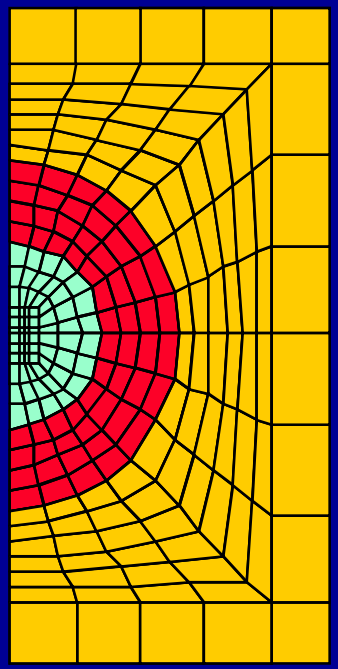


Conclusions: Permeability

- PCM is ~20 times less permeable than the ECM

ECM: ~9×10^{-16} m^4/Ns ⟶

PCM: ~0.4×10^{-16} m^4/Ns ⟶

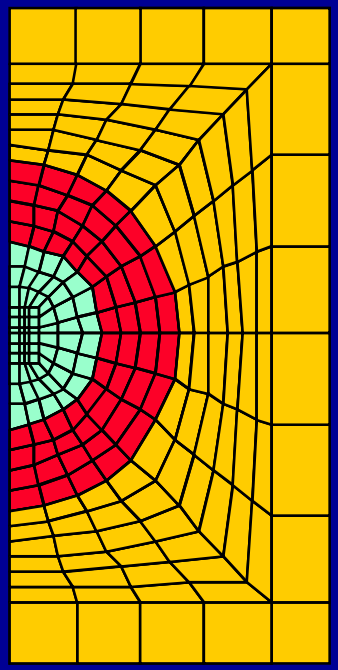


Conclusions: Poisson's ratio

- PCM and ECM have similar Poisson's ratio

ECM: ~0.04 →

PCM: ~0.04 →



Conclusions….

In cartilage:

PCM is a distinct tissue region with significantly different mechanical properties than the ECM

Conclusions….

With OA pericellular matrix undergoes degenerative changes similar to that of articular cartilage

- **Decrease in stiffness (~40%)**
- **Increase in permeability (2.5 times)**

HYPOTHESES

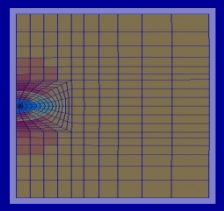
1. The mechanical properties of the PCM are altered with OA

2. **The PCM regulates the mechanical environment of chondrocytes in normal and OA cartilage**

3. Collagen type VI affects PCM stiffness, cartilage development, and progression of osteoarthritis

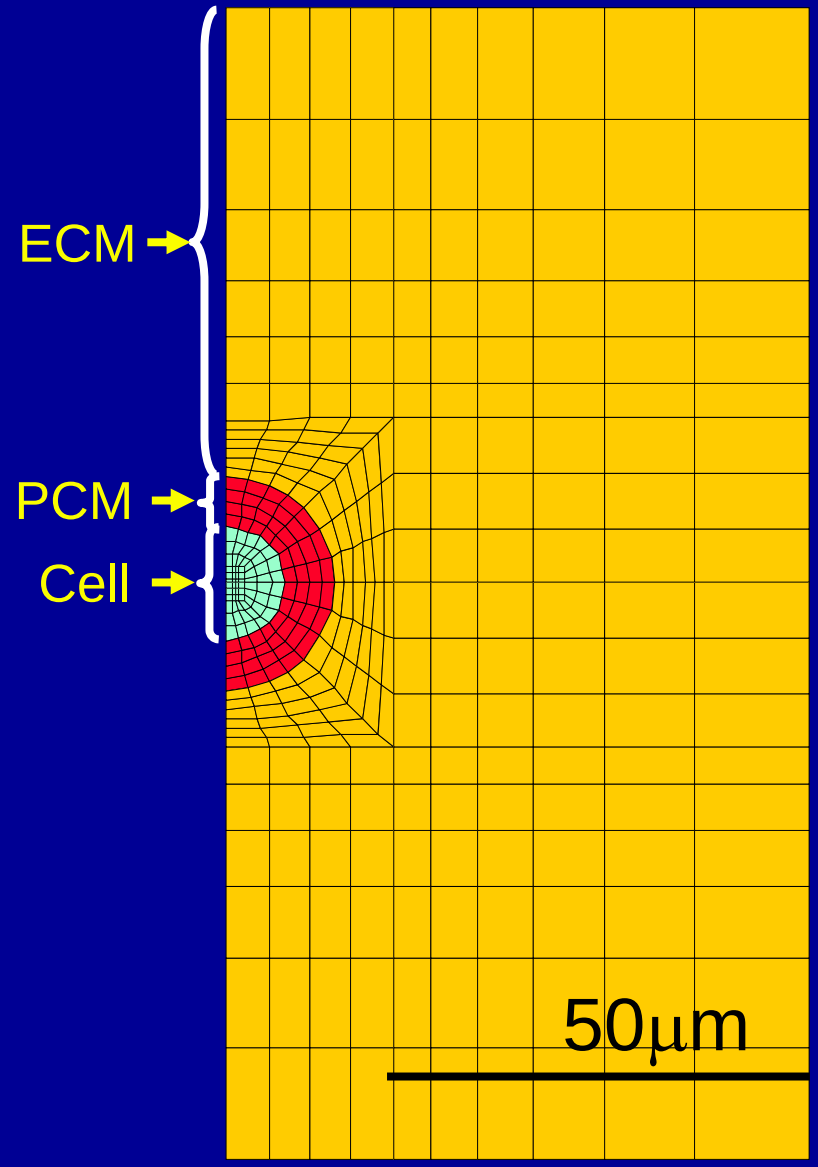
2 Material and Methods

- The Young's modulus, permeability and Poisson's ratio of normal and OA chondrons were used



- A biphasic FEM model of the CELL-PCM-ECM structure was developed to evaluate that biomechanical role of the PCM.

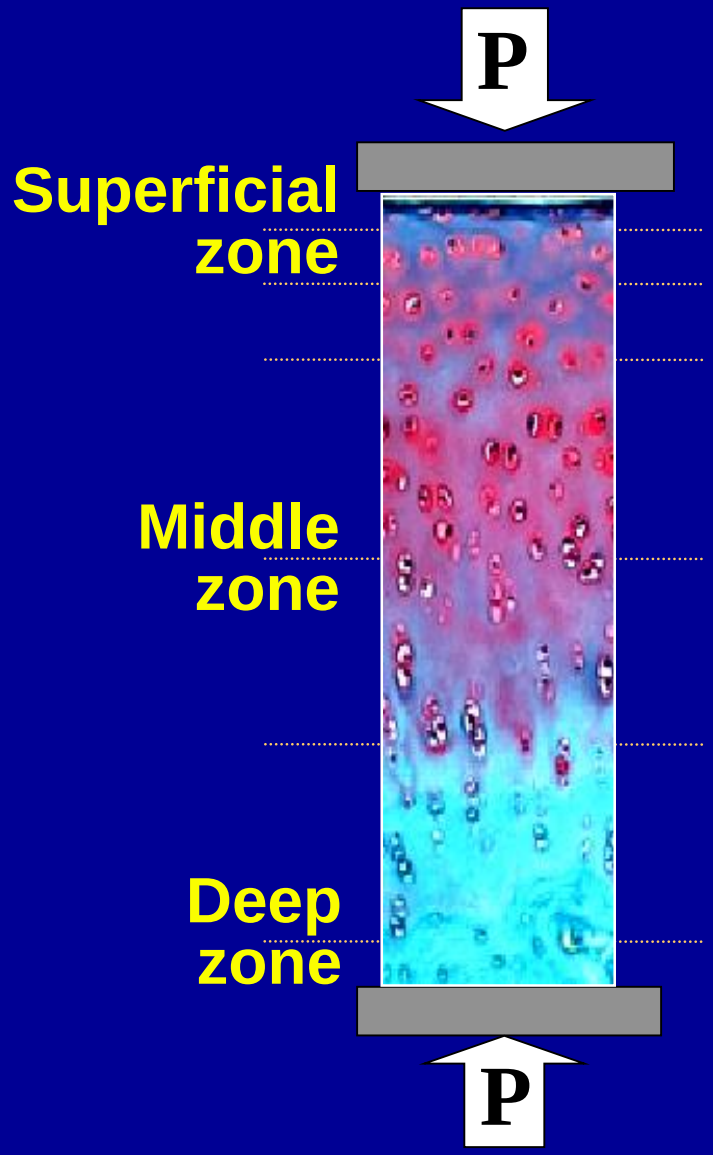
2 | Finite Element Model



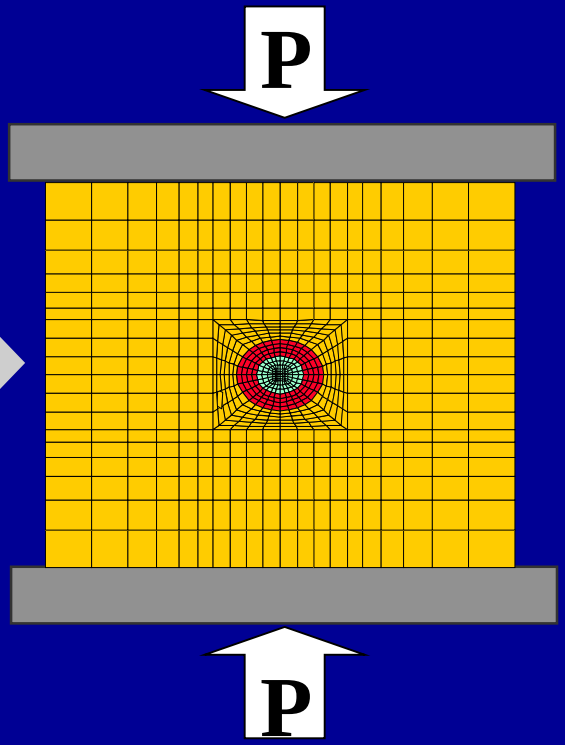
	PROPERTIES	CELL	PCM
Normal	E: kPa	0.36	40
	k×10^{-15}:m^4/Ns	4.2	0.04
	ν	0.4	0.04
OA	E: kPa	0.5	20
	k×10^{-15}:m^4/Ns	2.4	0.13
	ν	0.4	0.04

Koay et al., 2003, Knight et al., 2002, Trickey et al., 2000, Guilak et al 2000, Alexopoulos et al. 2004

2 | FEM: Compression tests



Unconfined compression



2 | FEM: Compression tests

Measured values:

A. STRAIN ENVIRONMENT

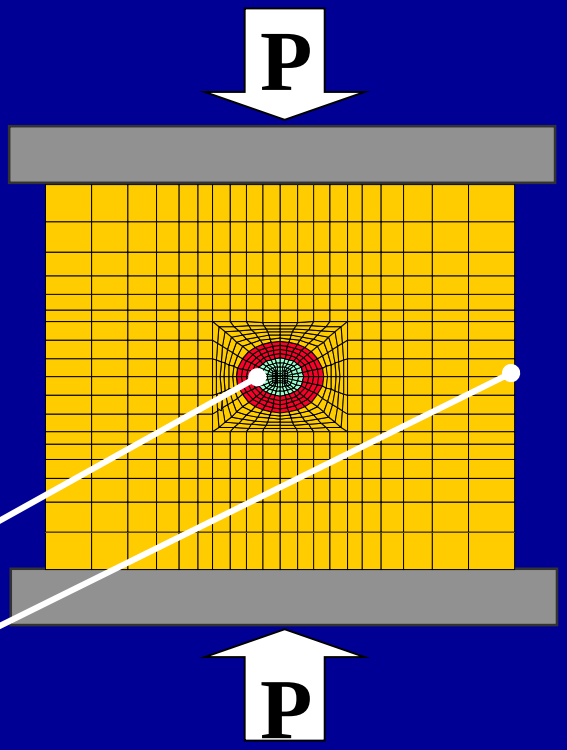
$$\text{Cell Strain Amplification} = \frac{\text{Cell Strain}}{\text{Tissue Strain}}$$

$$\text{Where: Cell strain} = \frac{\Delta(\text{Cell height})}{\text{Cell diameter}}$$

B. STRESS ENVIRONMENT

$$\text{Normalized Stress} = \frac{\sigma_{zz,\,cell}}{\sigma_{zz,\,block}\,(=P)}$$

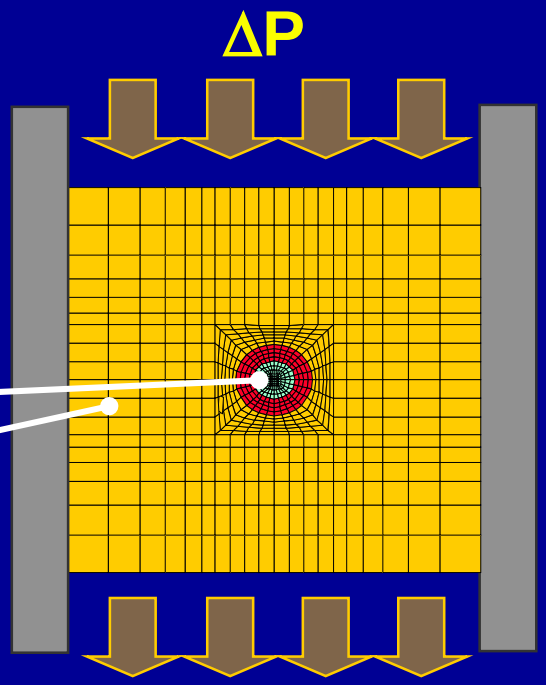
Unconfined compression



2 | FEM: Flux tests

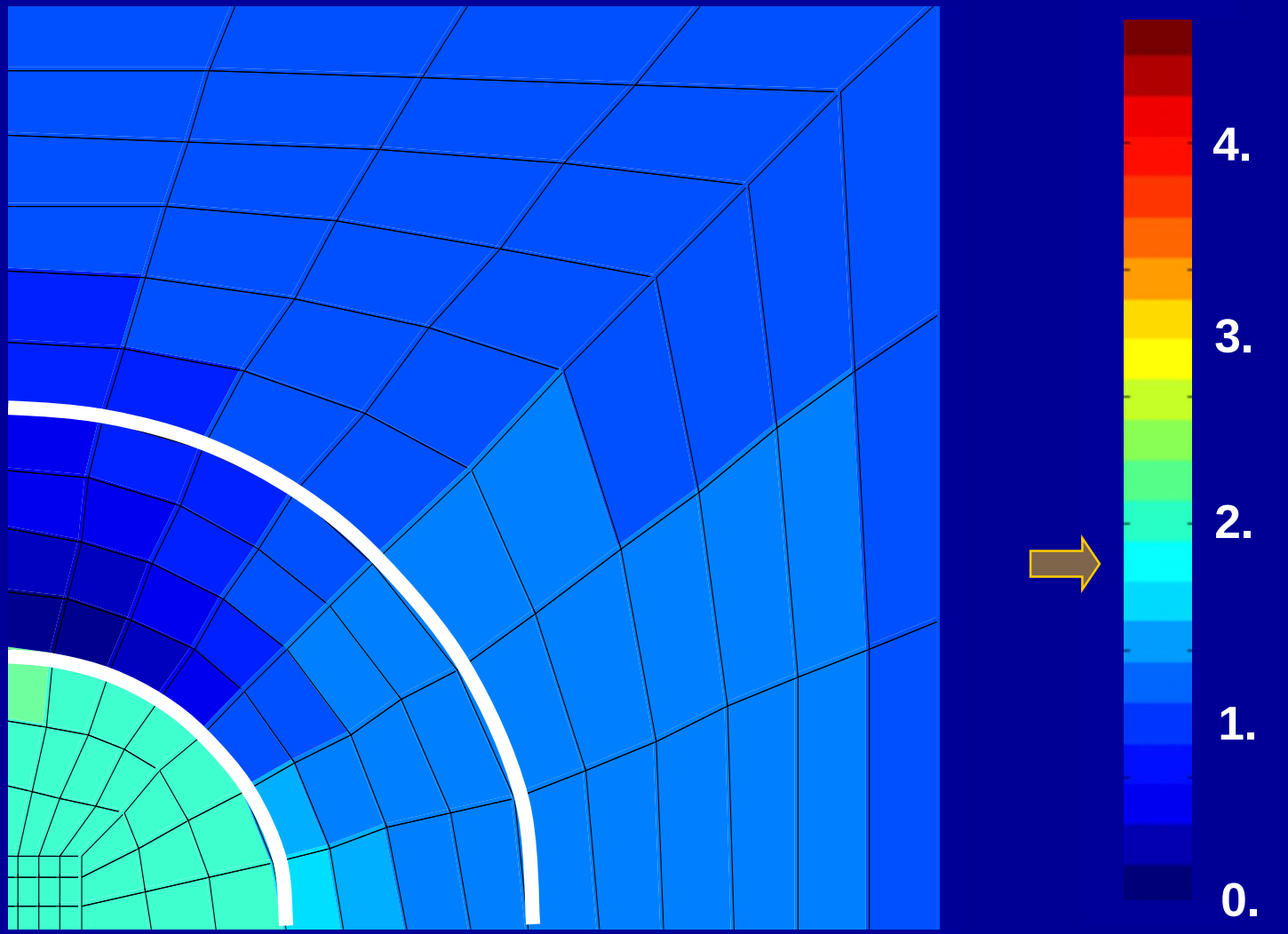
C. FLUX ENVIRONMENT

$$\text{Normalized Flux} = \frac{\text{flux}_{z,\,\text{cell}}}{\text{flux}_{z,\,\text{far field}}}$$



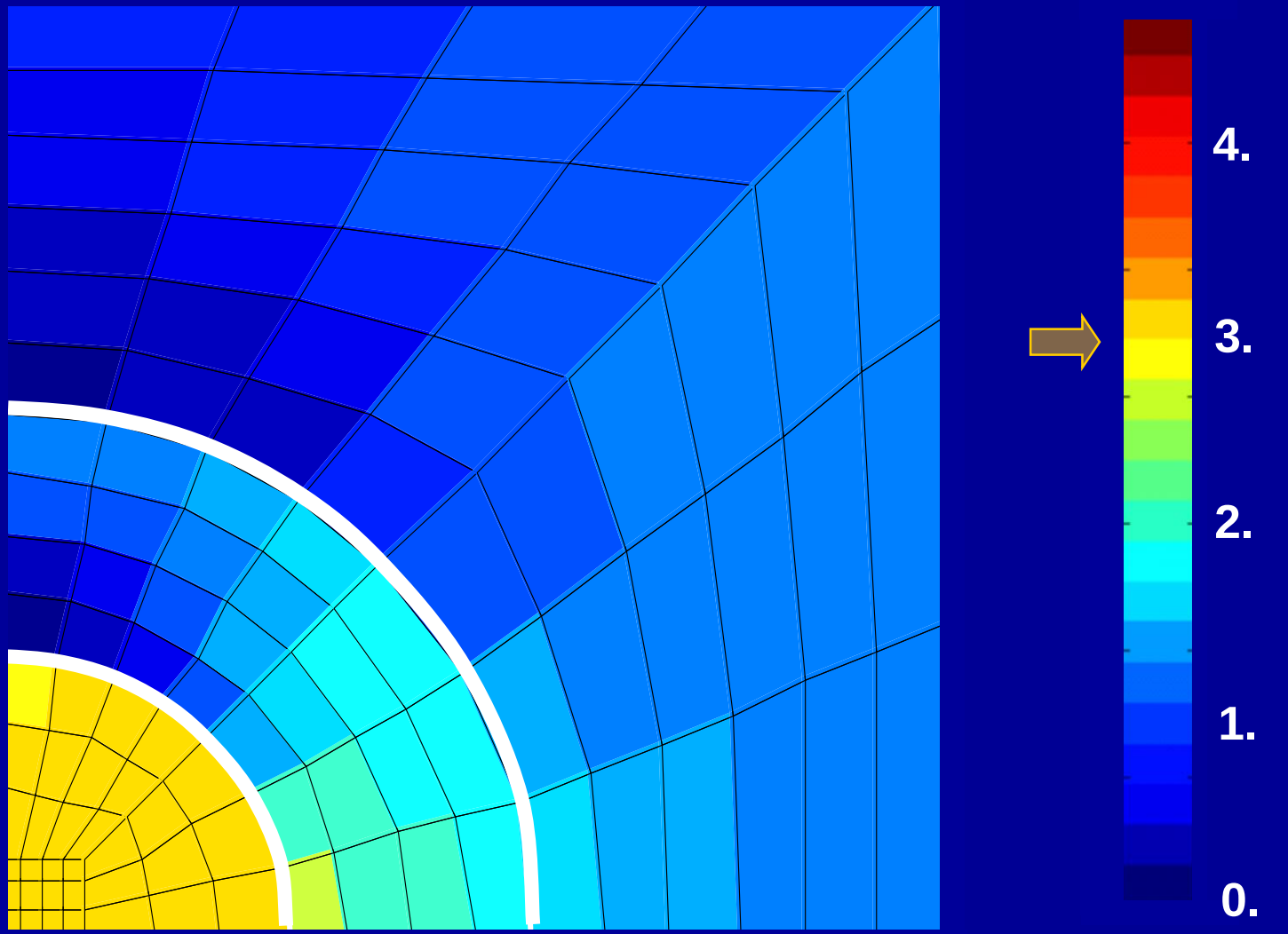
2 | Results: Cell strain amplification

NORMAL condition without PCM



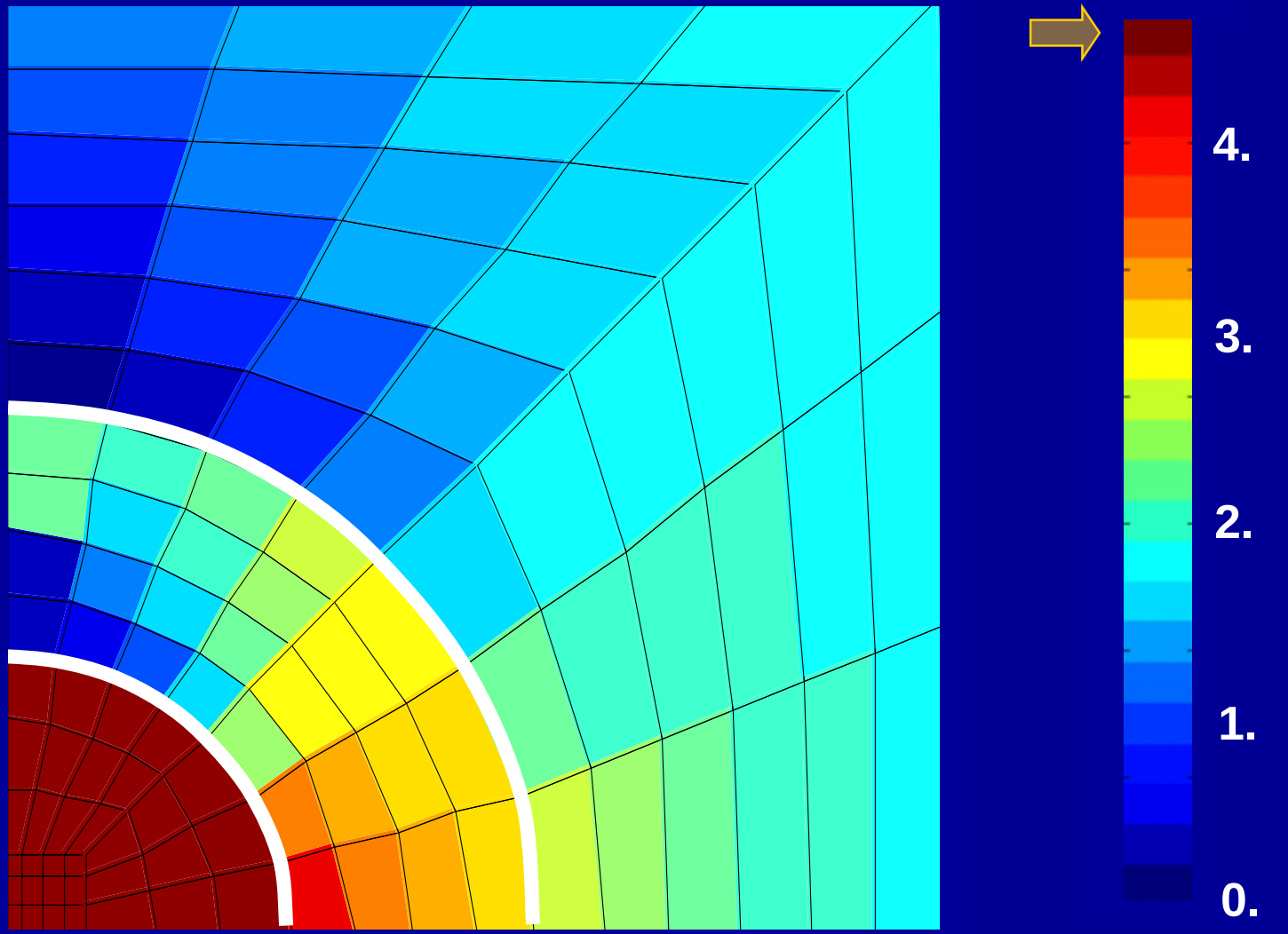
2 | Results: Cell strain amplification

NORMAL condition with PCM



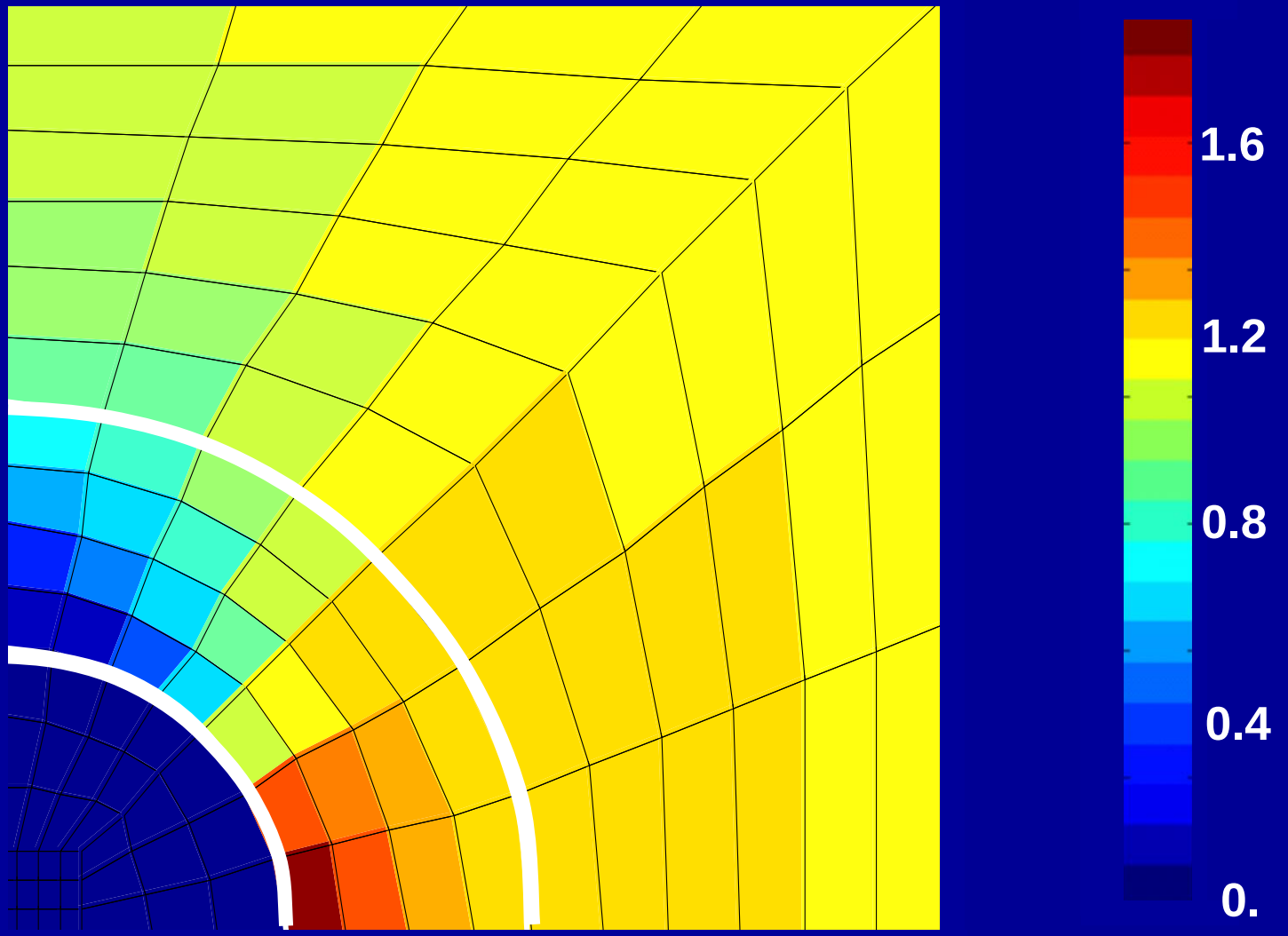
2 | Results: Cell strain amplification

OA condition with PCM



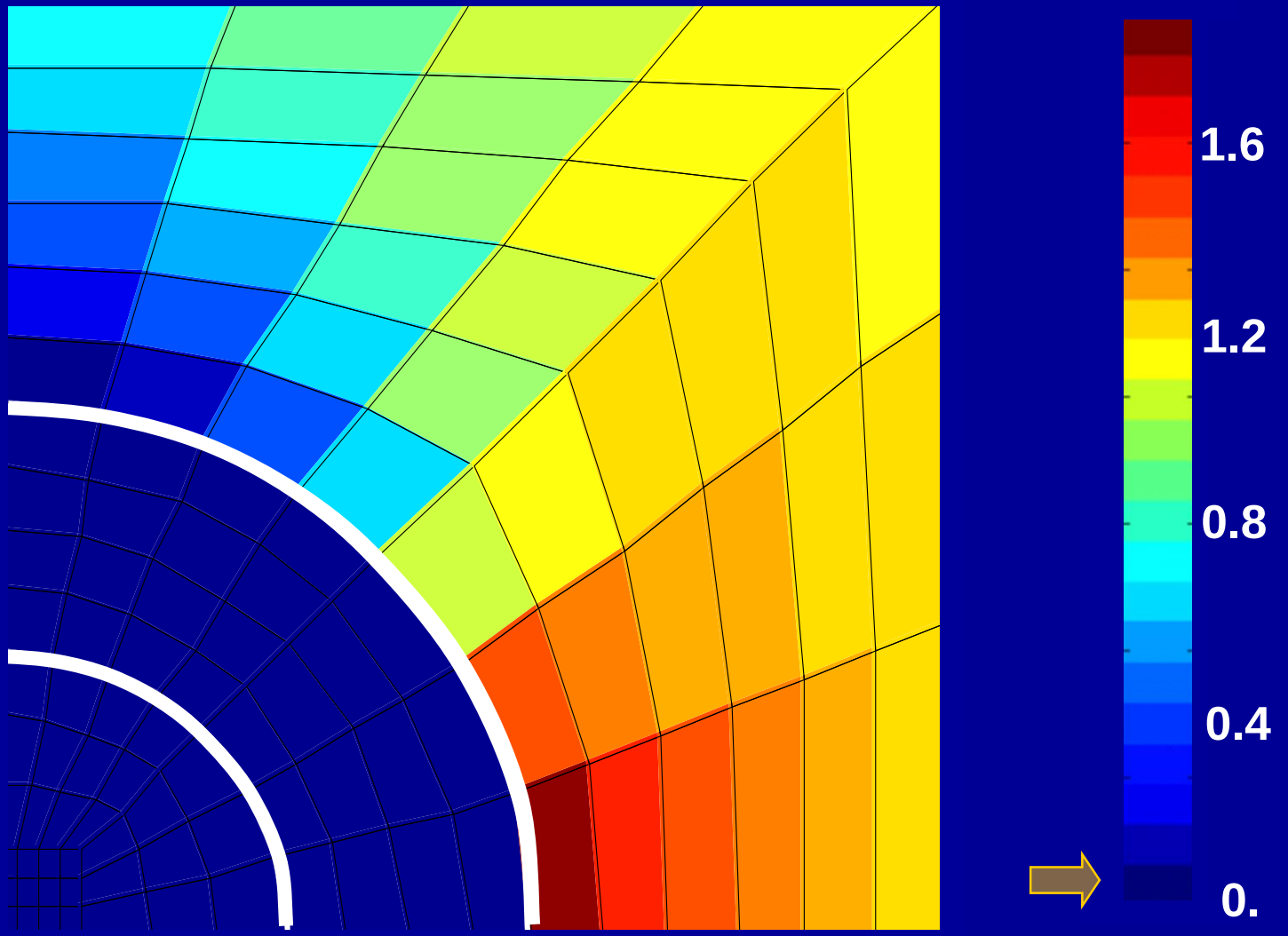
2 | Results: Normalized Stress σ_{zz}

NORMAL condition without PCM

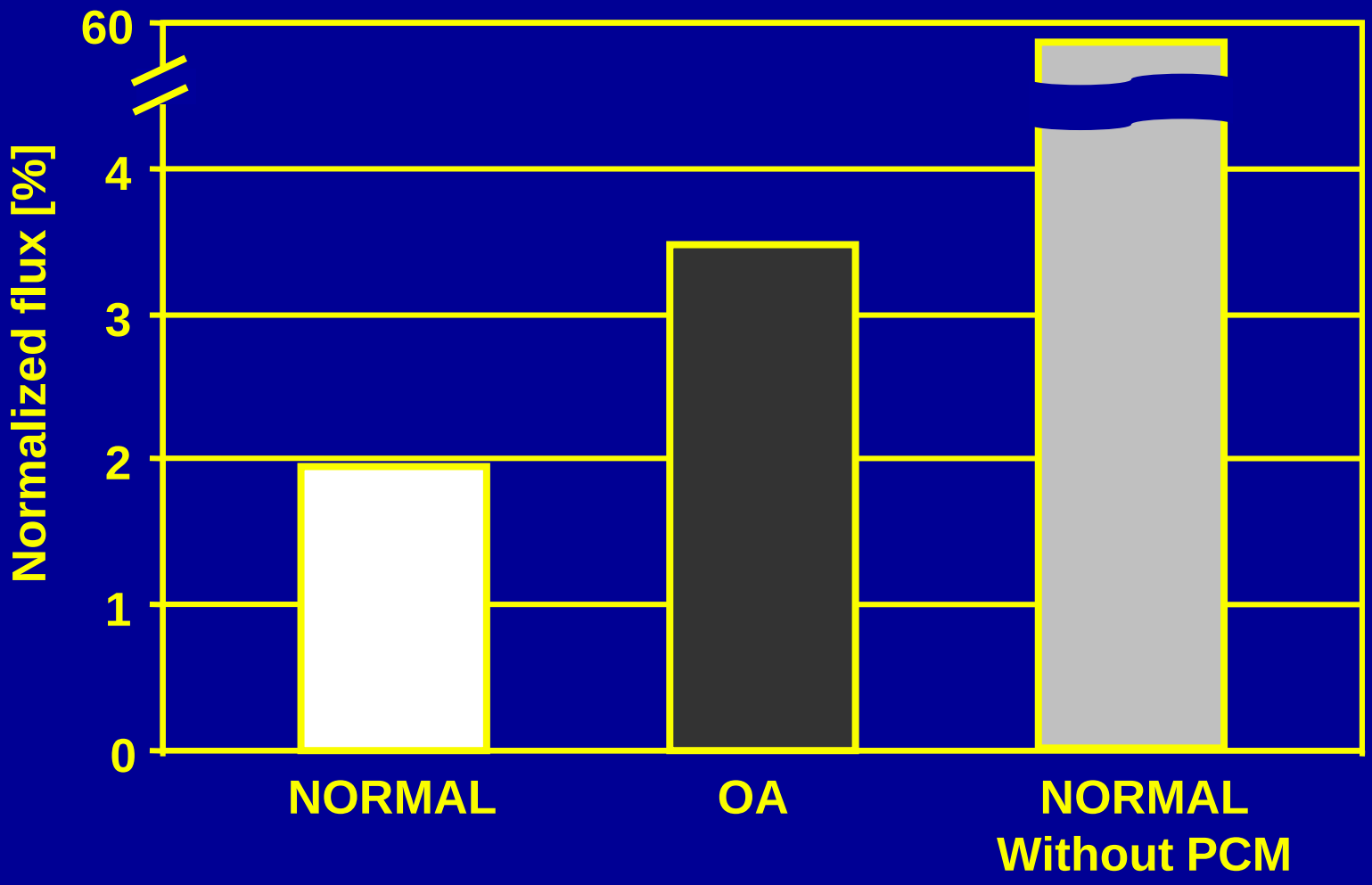


2 | Results: Normalized Stress σ_{zz}

NORMAL condition with PCM



2 | Results: Fluid Flux



2 | Conclusions

The PCM does have a dramatic influence on the chondrocyte mechanical environment

IN NORMAL CARTILAGE THE PCM:

1. **protects the cell from the local fluid fluxes**
2. **amplifies the tissue strains**
3. **has a significant stress shielding effect**

2 | Conclusions

The PCM does have a dramatic influence on the chondrocyte mechanical environment

IN OA CARTILAGE:

1. **The cell compressive strains are increased ~100% (injurious load?)**
2. **The cells experience 50% higher local fluid velocities**

HYPOTHESES

1. The mechanical properties of the PCM are altered with OA

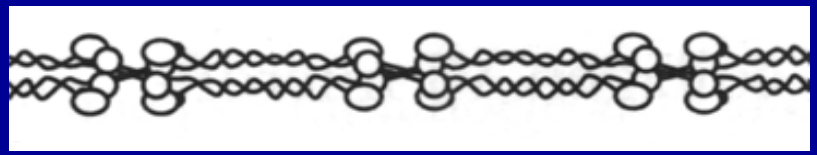
2. The PCM regulates the mechanical environment of chondrocytes in normal and OA cartilage

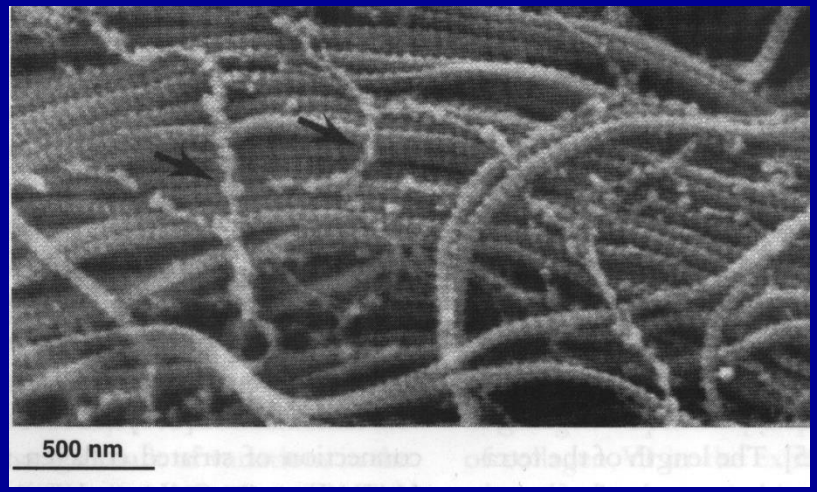
3. **Collagen type VI affects PCM stiffness, cartilage development, and progression of osteoarthritis**

3 | Collagen VI

STRUCTURE

beaded filament





Keene et al., 1998. (Reproduced from The Journal of Cell Biology by copyright permission of The Rockefeller University Press)

INTERACTIONS

MATRIX - MATRIX

Biglycan, Decorin, Fibromodulin, Hyaluronan, Fibronectin, Perlecan, Collagen II (via matrilin-1)

Bidanset et al. 1992, McDevitt et al. 1991, Specks et al. 1992, Tillet et al. 1994, Wiberg et al. 2001, Wiberg et al. 2003

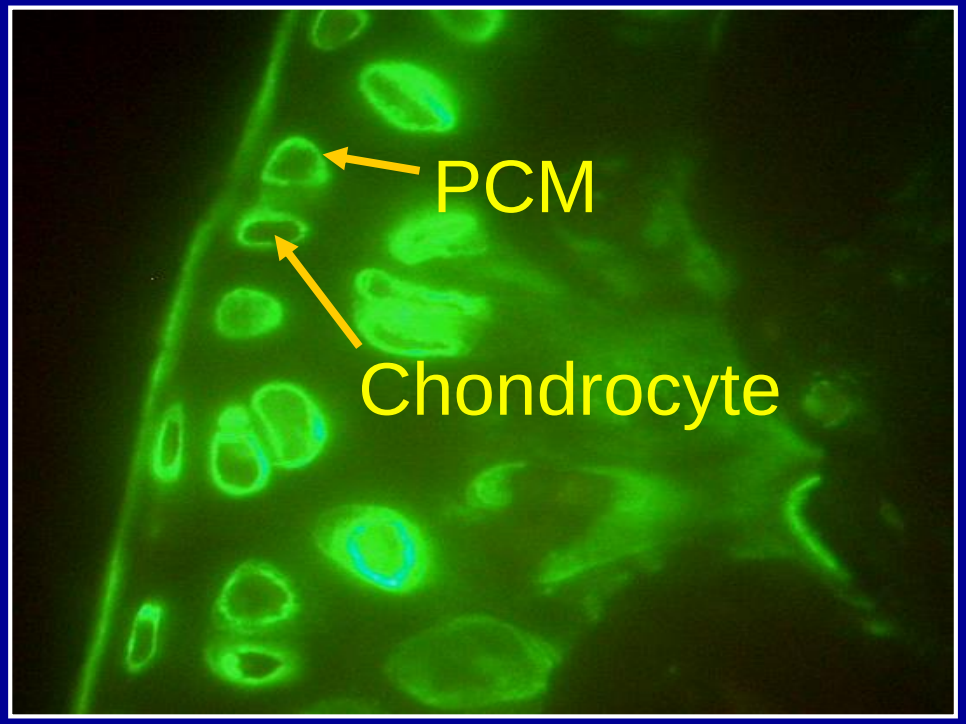
CELL – MATRIX

Integrin receptors

Doane et al. 1998, Doane et al. 1992, Pfaff et al. 1993, Salter et al. 1992

3 | Collagen VI and cartilage

- **May anchor the cell to the PCM and the PCM to ECM** (Buckwalter et al. 1998, Keene et al. 1998, Marcelino et al. 1995, Poole et al. 1992, Sherwin et al. 1999)
- **May enhance the mechanical properties of PCM** (Poole et al. 1997)



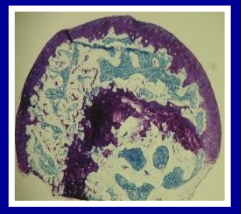
3 | Collagen VI and development

- **Type VI collagen may provide a scaffold for osteoblasts, preosteoblasts, and chondrocytes to proceed to osteochondral ossification** (Tanaka et al. 2003)
- **Type VI collagen regulates the mesenchymal cell proliferation *in vitro*** (Atkinson et al. 1996)
- **Type VI collagen is linked to the early events of chondrocyte differentiation** (Quarto et al. 1993)

3 Materials and Methods

•Collagen type VI knockout mice were used. Genotyping was performed to confirm the phenotype

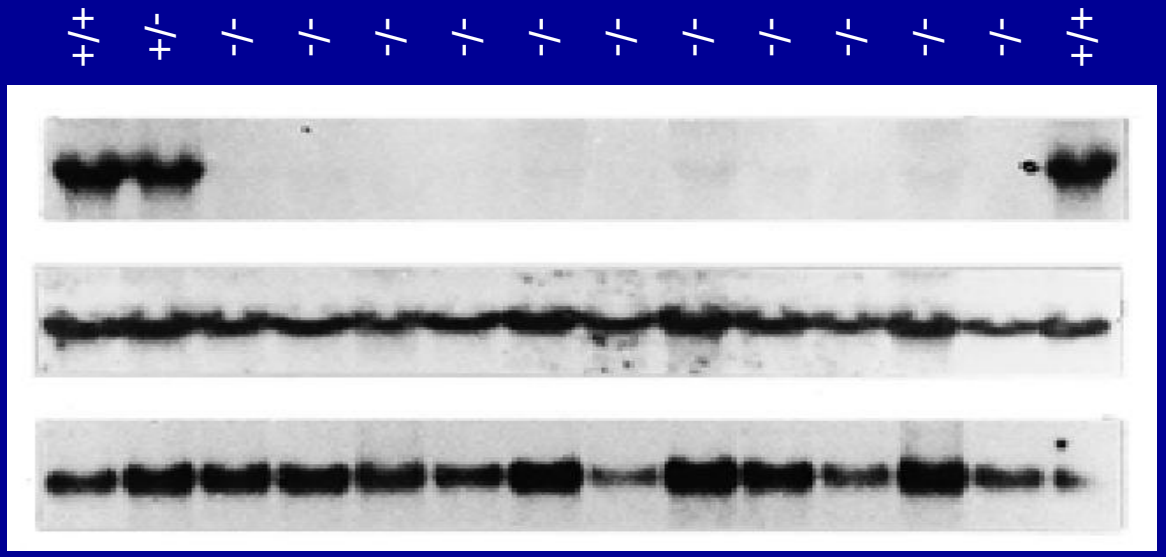
•The mechanical properties of the PCM from wild type and knockout mice were compared



•Histological analysis of the femoral head was performed to quantify developmental and osteoarthritic changes

Collagen VI knockout mice

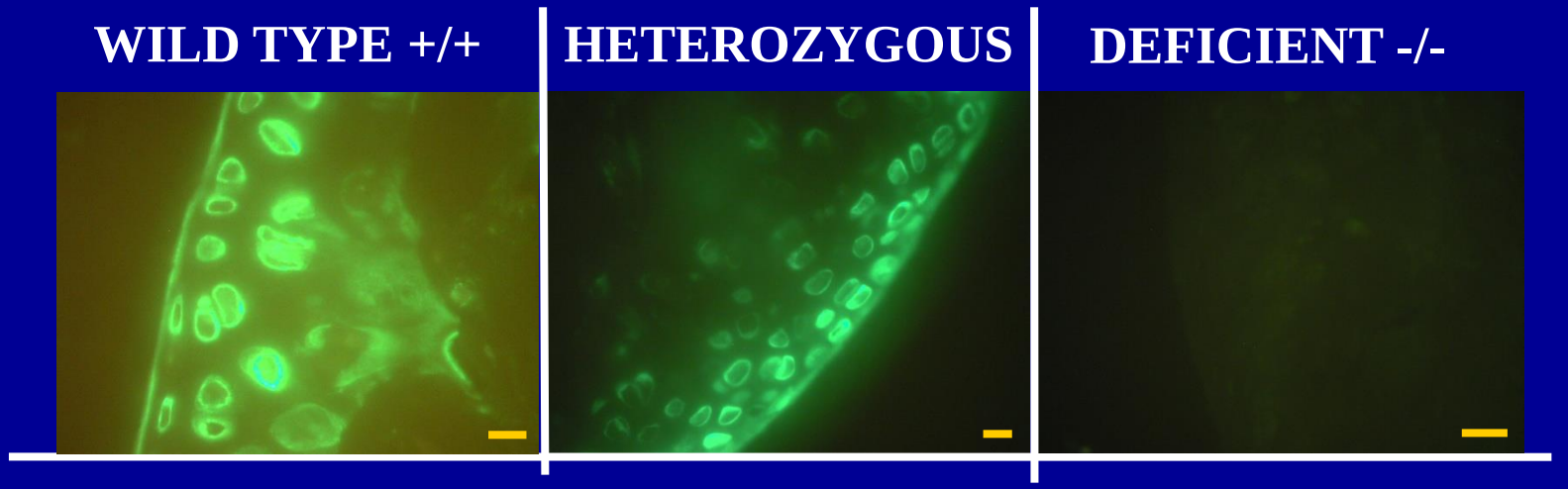
Bonaldo et al [1998] inactivated the col6α1 gene in mice. The homozygous mutant lacked Collagen VI.



Northern blot analysis reveals no α1(VI) mRNA in the -/-

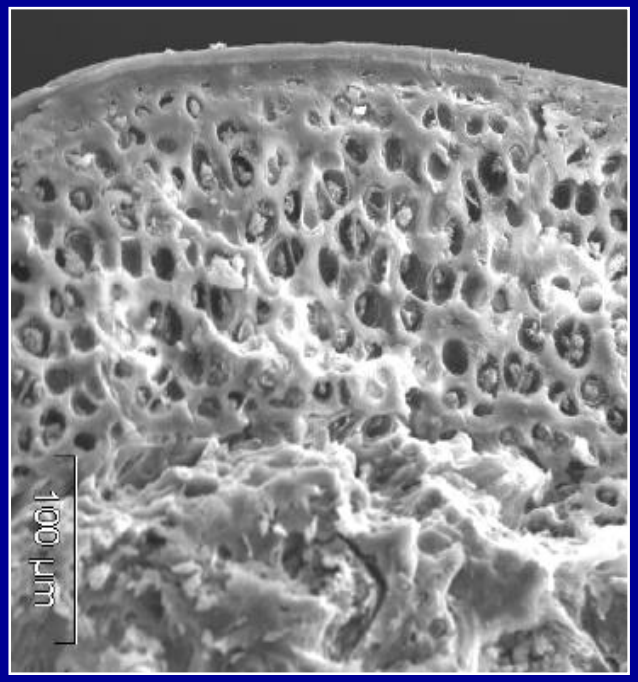
Collagen VI knockout mice

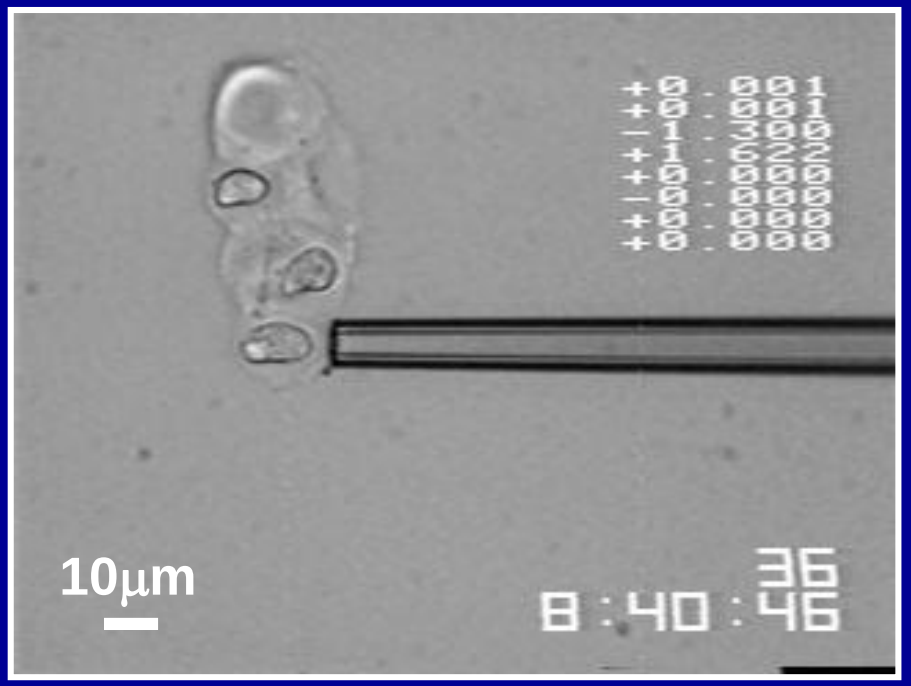
Bonaldo et al [1998] inactivated the col6α1 gene in mice. The homozygous mutant lacked Collagen VI.



Bars: 20μm

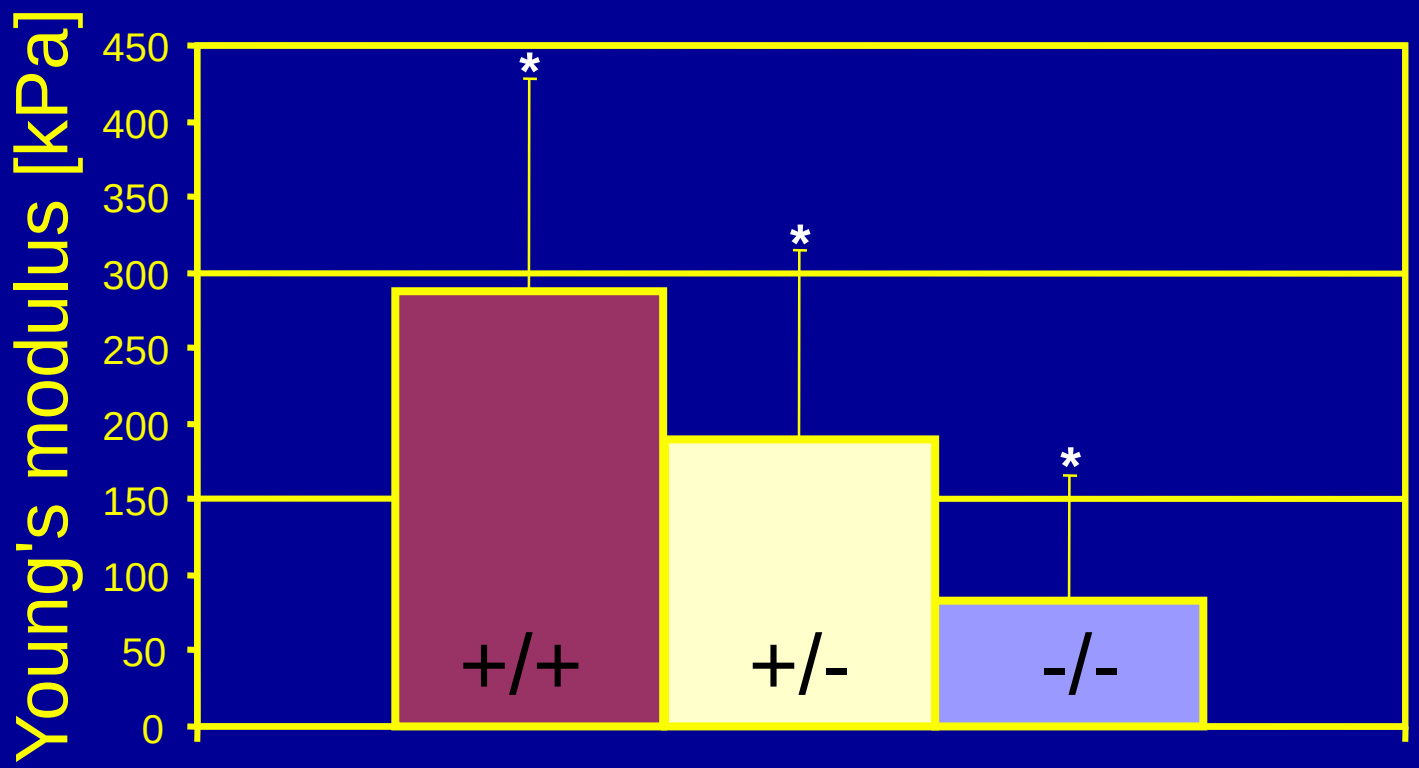
Chondron Isolation from mouse cartilage





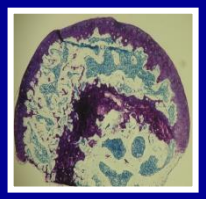
Chondrons were isolated from wild type (+/+), heterozygous (+/-), and homozygous (-/-) mice

Collagen VI affects the mechanical properties of the PCM

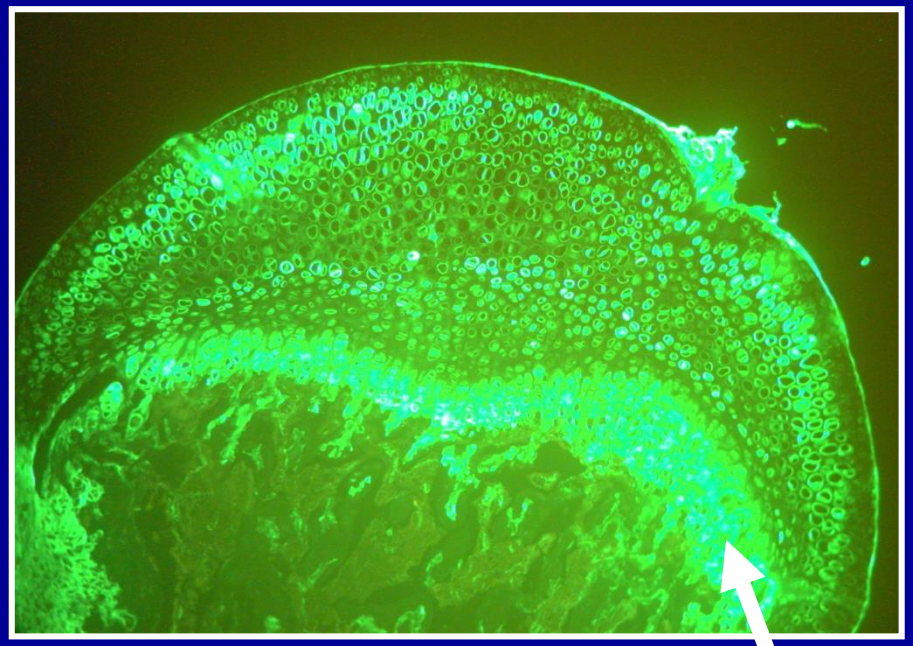


1 month old mice

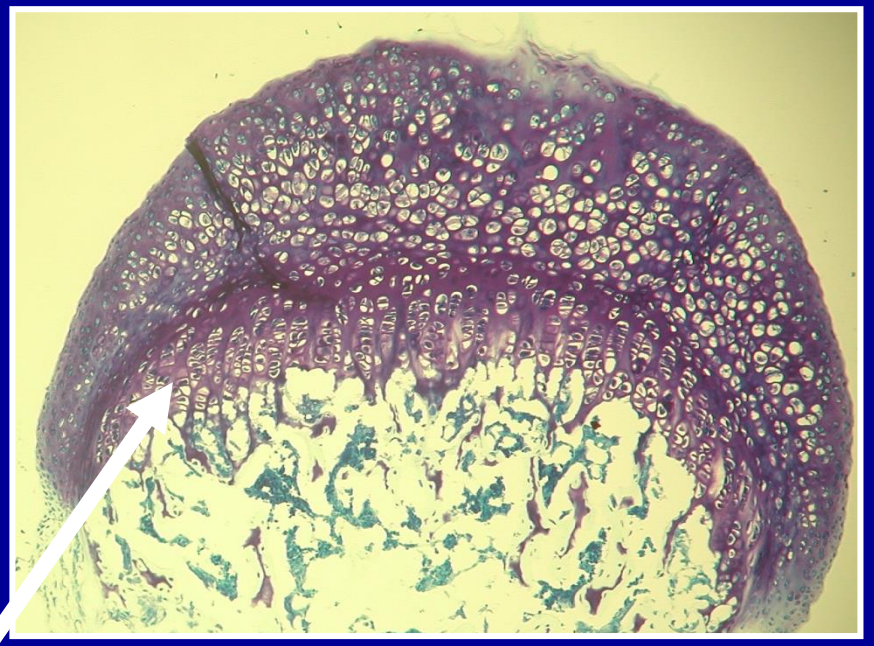
*p<0.001, n=93 chondrons from N=26 donors, one month old



Collagen VI and development

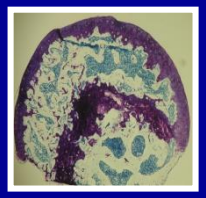


1 month old: Collagen VI



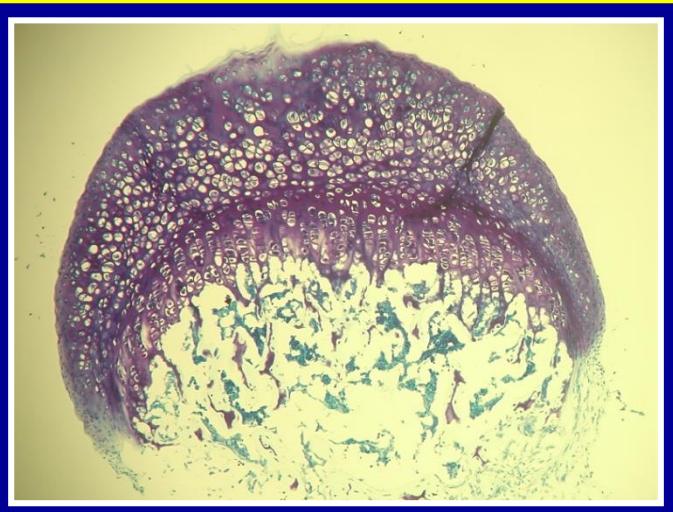
1 month old: Toluidine blue

Collagen VI is significantly upregulated in the growth plate of 1 month old mice

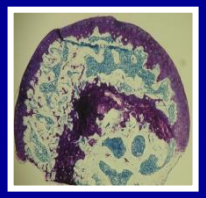


Developmental grading

	Grade
Cartilage overlying growth plate overlying bone	**0**
Secondary ossification starts with total area < 10%	**1**
Secondary ossification in progress with total area < 50%	**2**
Secondary ossification in progress with total area > 50%	**3**
Secondary ossification completed with uncalcified areas < 10%	**4**
Cartilage overlying bone	**5**



Rivas et al. 2002



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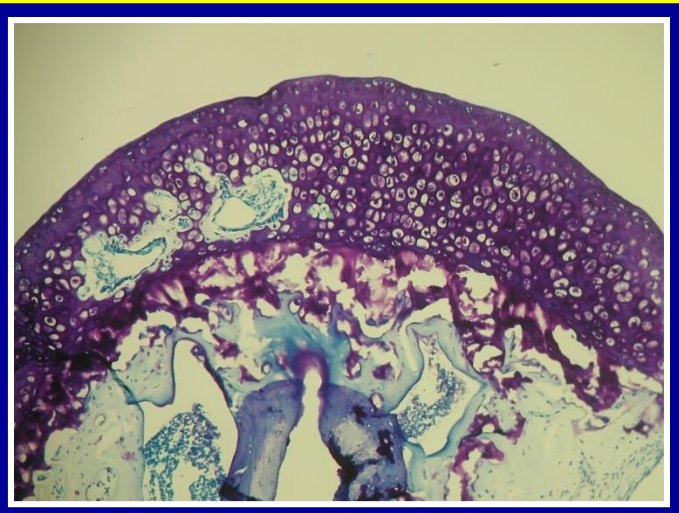
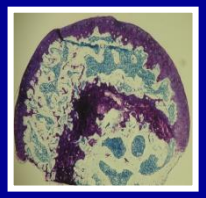


Rivas et al. 2002



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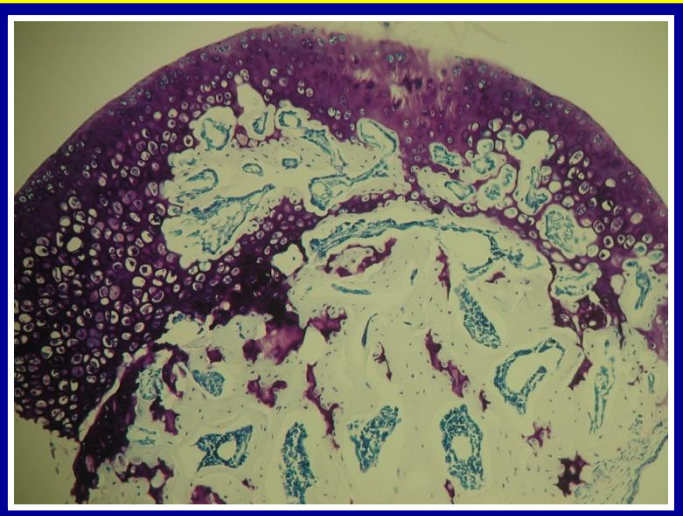

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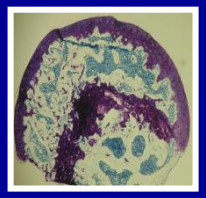

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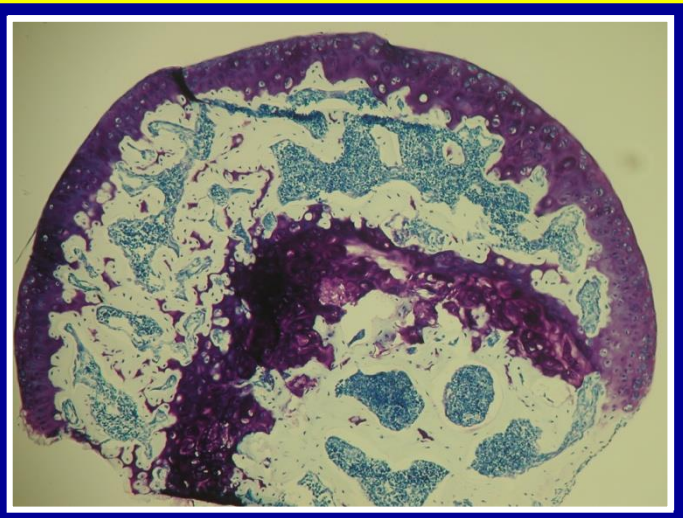
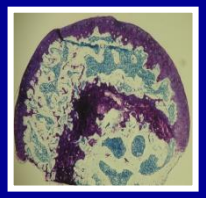


Rivas et al. 2002



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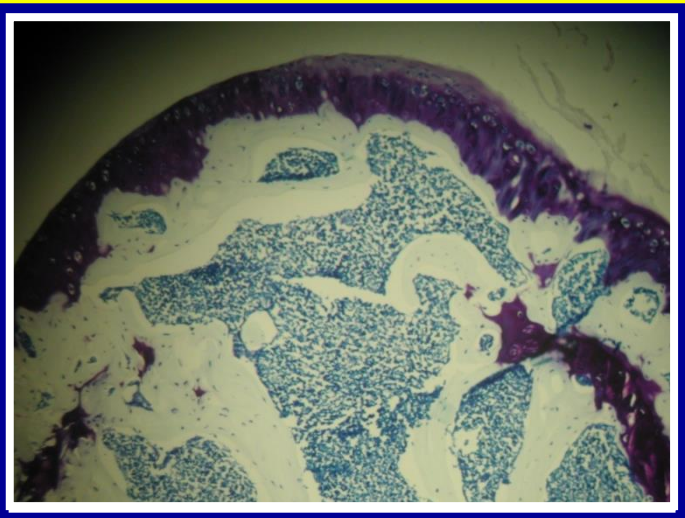
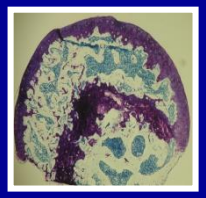


Rivas et al. 2002



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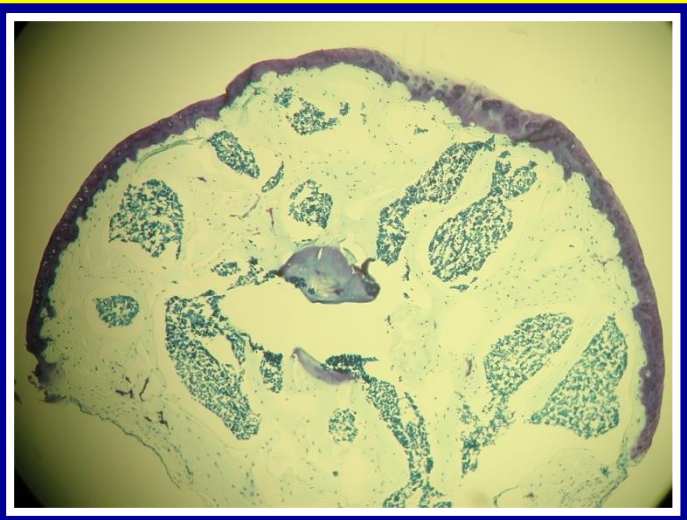
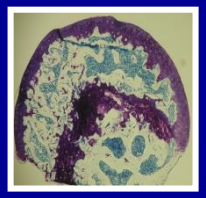
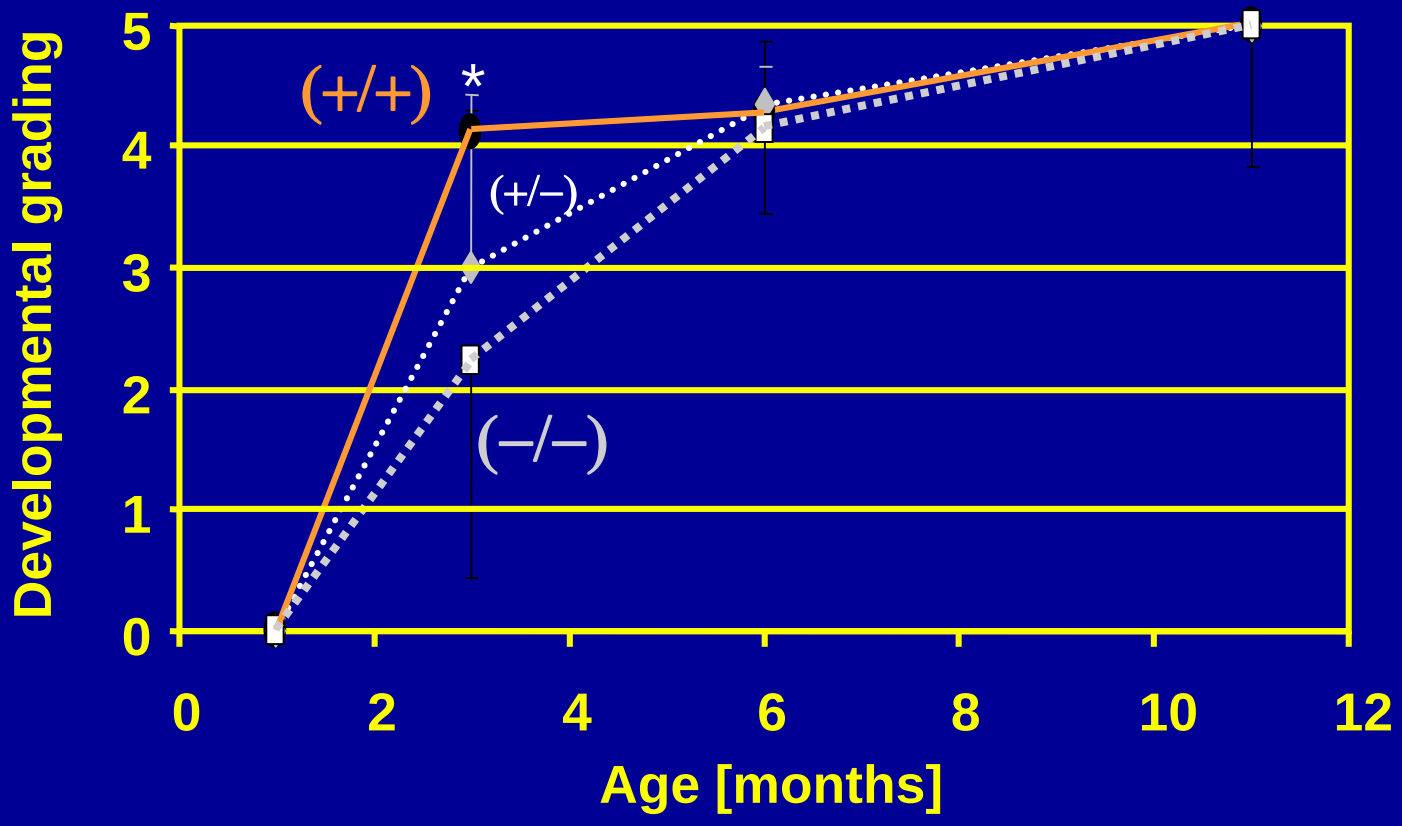


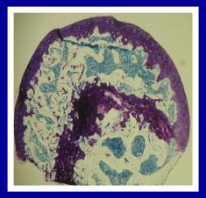
Rivas et al. 2002



Development is slower in the 3 months old knockout mice



*p<0.04, N=57



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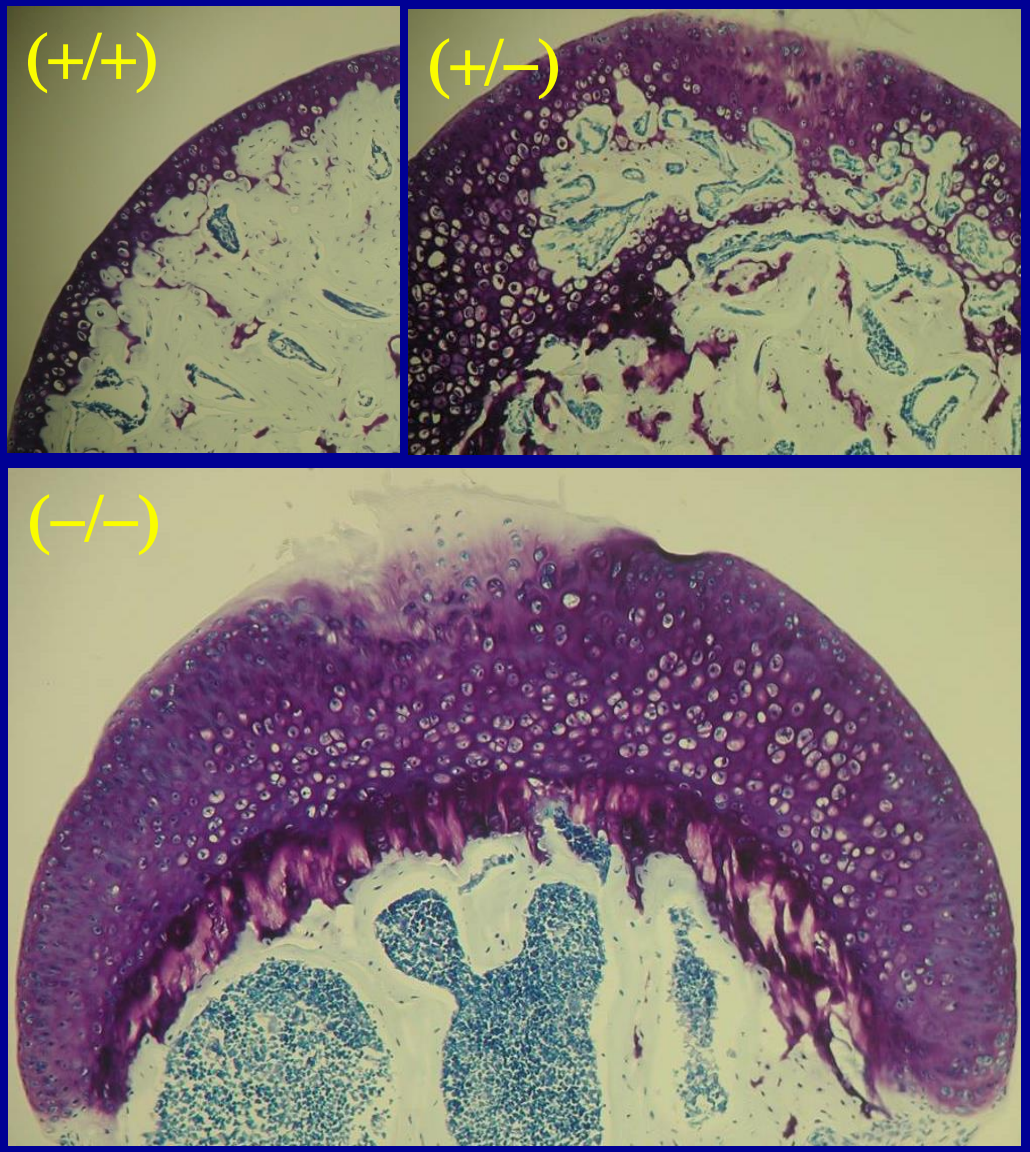
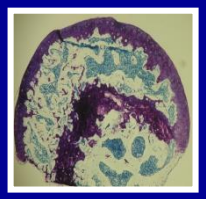
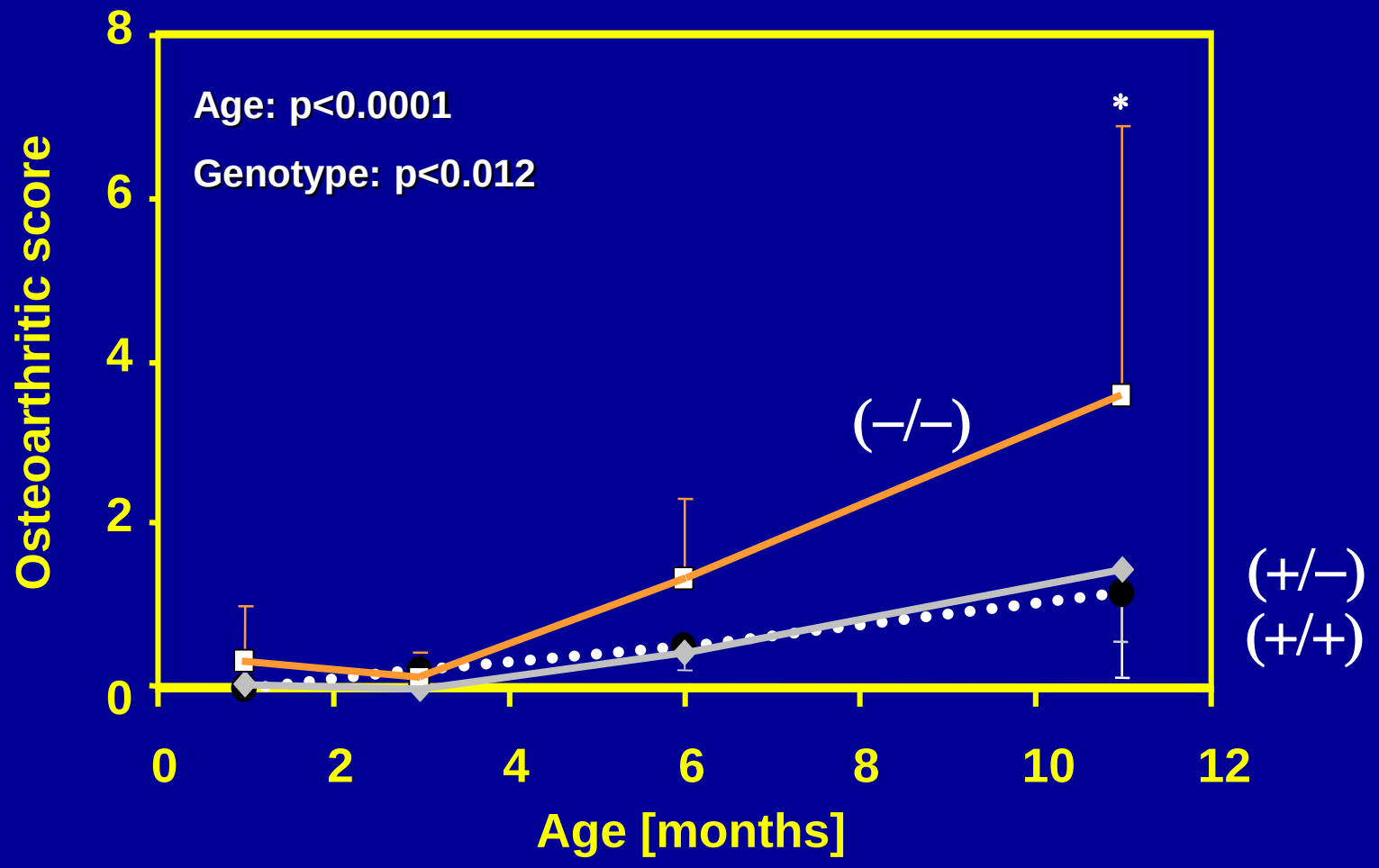
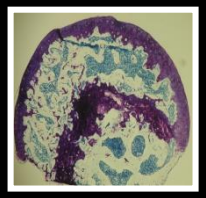




Mild osteoarthritic characteristics are increased in the knockout mice



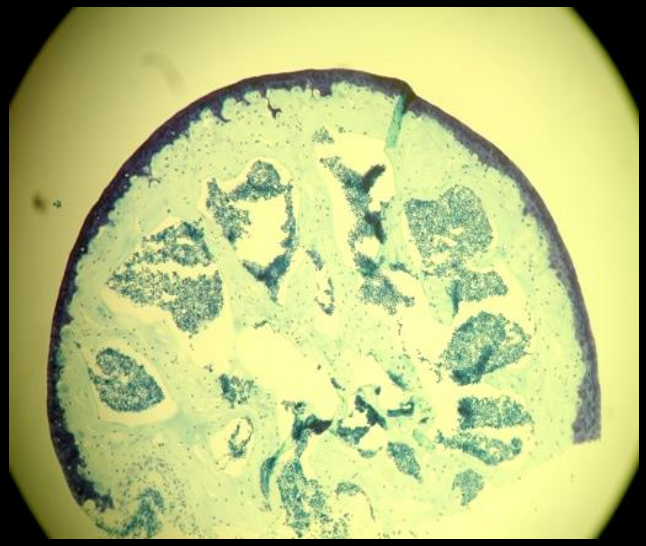
N=57

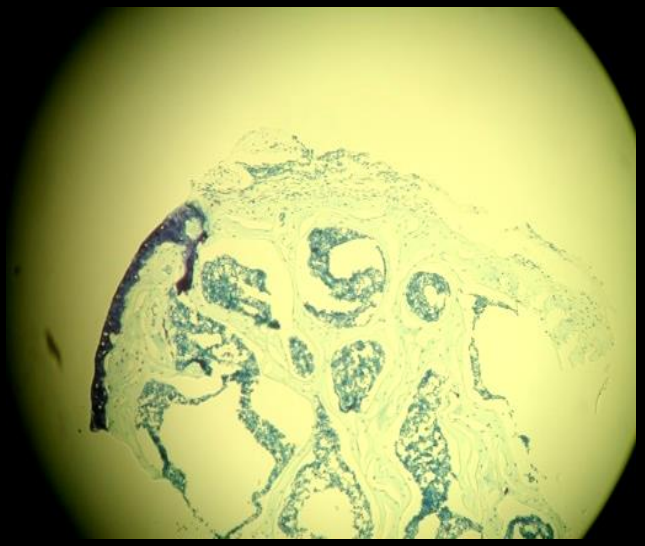


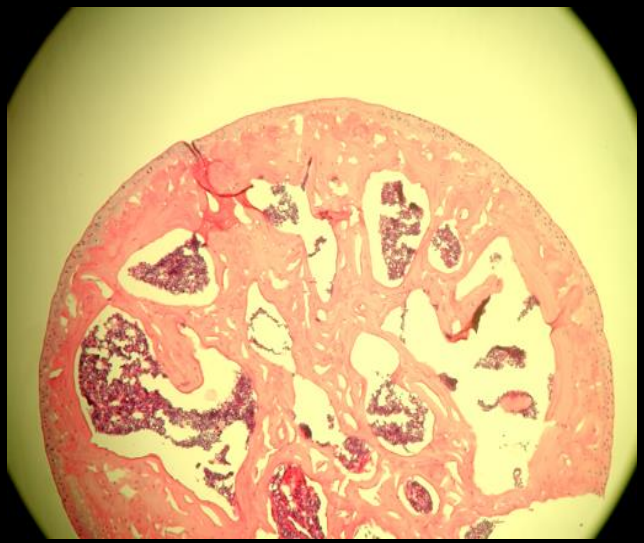
Mild osteoarthritic characteristics are increased in the knockout mice

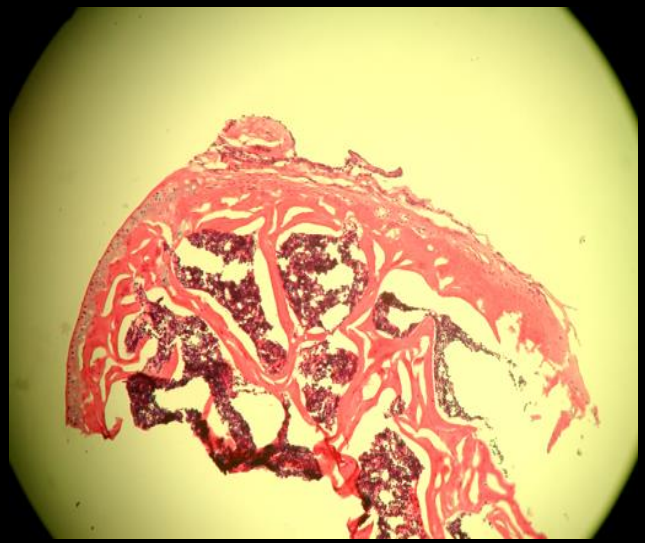
Col6a1(+/+)

Col6a1(-/-)









3 Collagen VI and chondrons

Collagen type VI plays a major role in the PCM stiffness.

- **Absence of type VI collagen results in a 3 fold decrease of the PCM stiffness**

Collagen VI deficiency does not prevent formation of chondrons

3 Collagen VI and development

Absence of collagen type VI results in slower secondary ossification progress

- **Type VI collagen may provide a scaffold in the growth plate for the osteochondral ossification to take place**

3 Collagen VI and Osteoarthritis

Absence of collagen type VI results in mild osteoarthritic characteristics.

- **Abnormal mechanical loads due to compromised PCM stiffness at 1 month old mice may result in the OA changes later in life**

GENERAL CONCLUSIONS

1. The mechanical properties of the PCM are altered with osteoarthritis

2. PCM regulates the micromechanical environment of cells. With OA, chondrocytes might be exposed to injurious compressive loads

3. Collagen type VI affects PCM stiffness, cartilage development, and progression of osteoarthritis. It does not affect chondron formation

ACKNOWLEDGEMENTS

My PhD committee

- Dr. Farshid Guilak
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- Dr. George Truskey
- Dr. Robert Hochmuth

Undergraduate Students

- Gregory Williams (UCSF)
- Jason Perera (Tufts University)
- Clint Walker
- Larry Martin (Morehouse College)

Other Collaborators

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- Dr. Beverley Fermor
- Dr. Hani Awad (Rochester Univ.)
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- Maureen Upton
- Bob Nielsen
- Steve Johnson

Duke University Orthopaedic Bioengineering Laboratory

NIH AG15768: "VISCOELASTIC PROPERTIES OF NORMAL AND OA CHONDRONS"

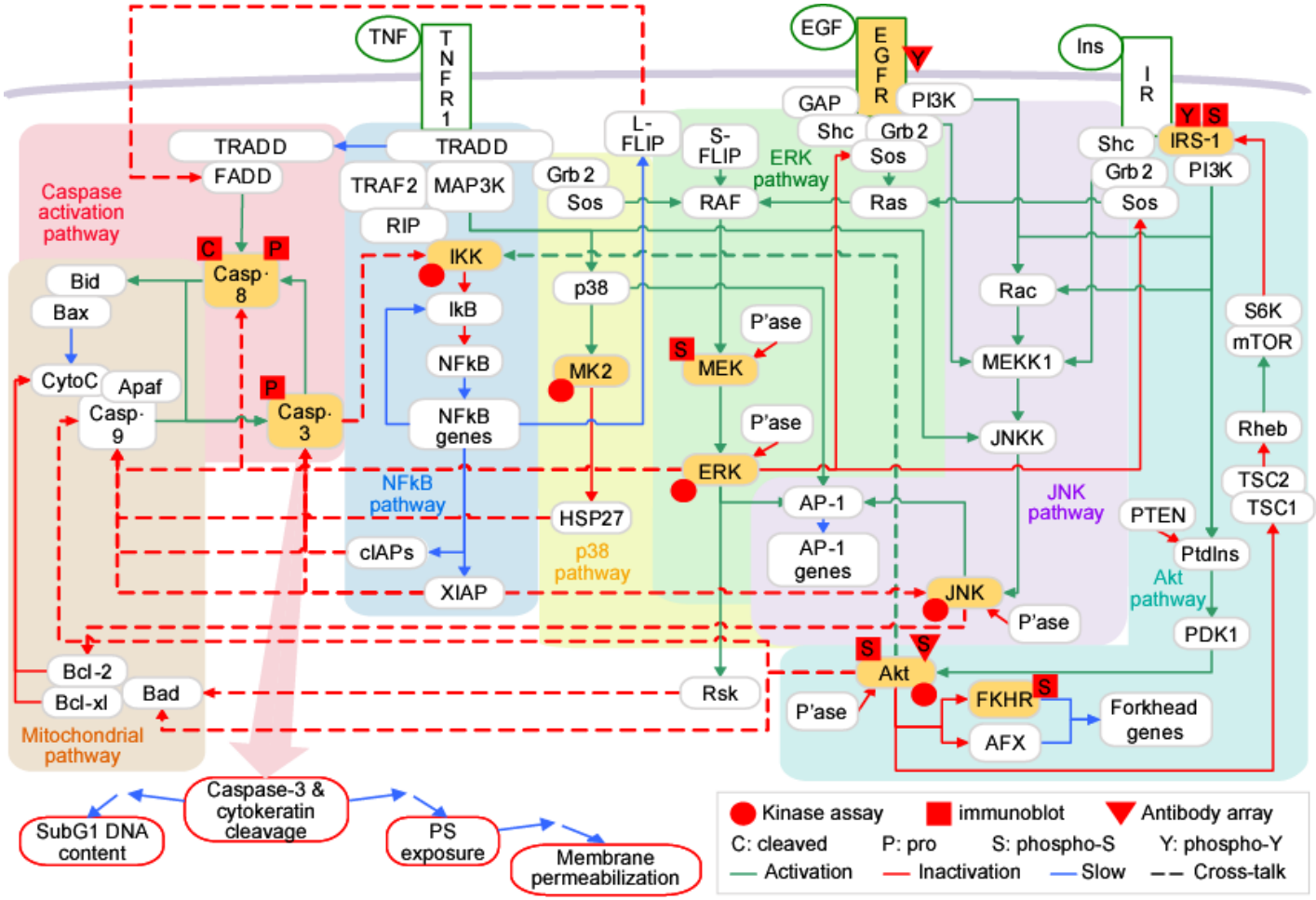
FUTURE WORK:

Autocrine Crosstalk in the response of chondrocytes to TNF & IL-1

Understanding the EGF, TNF, Insulin pathway

The MOAD Dataset - Janes, Gaudet, Albeck and Nielsen

HT29 - Human colon carcinoma cell lines



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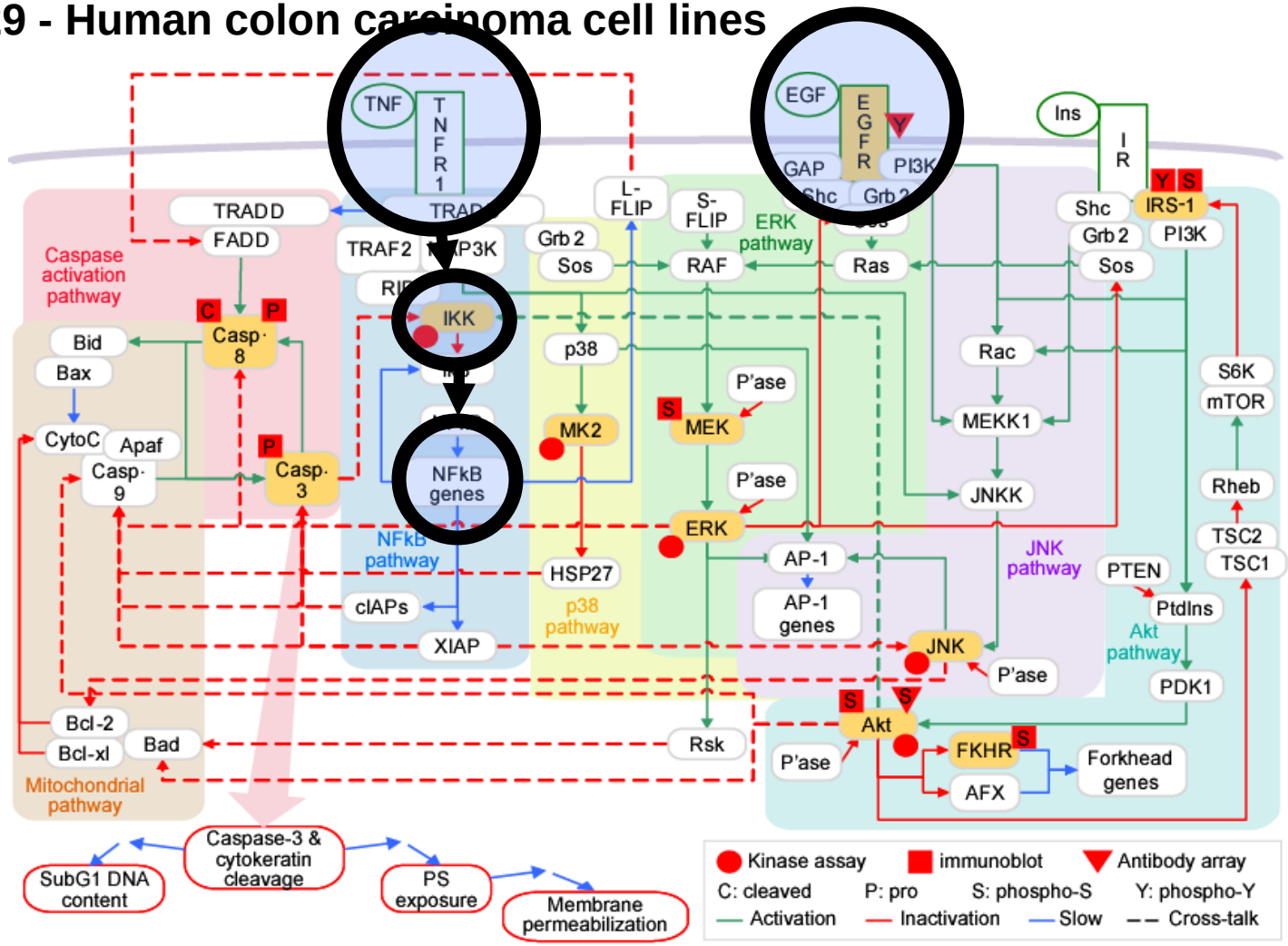
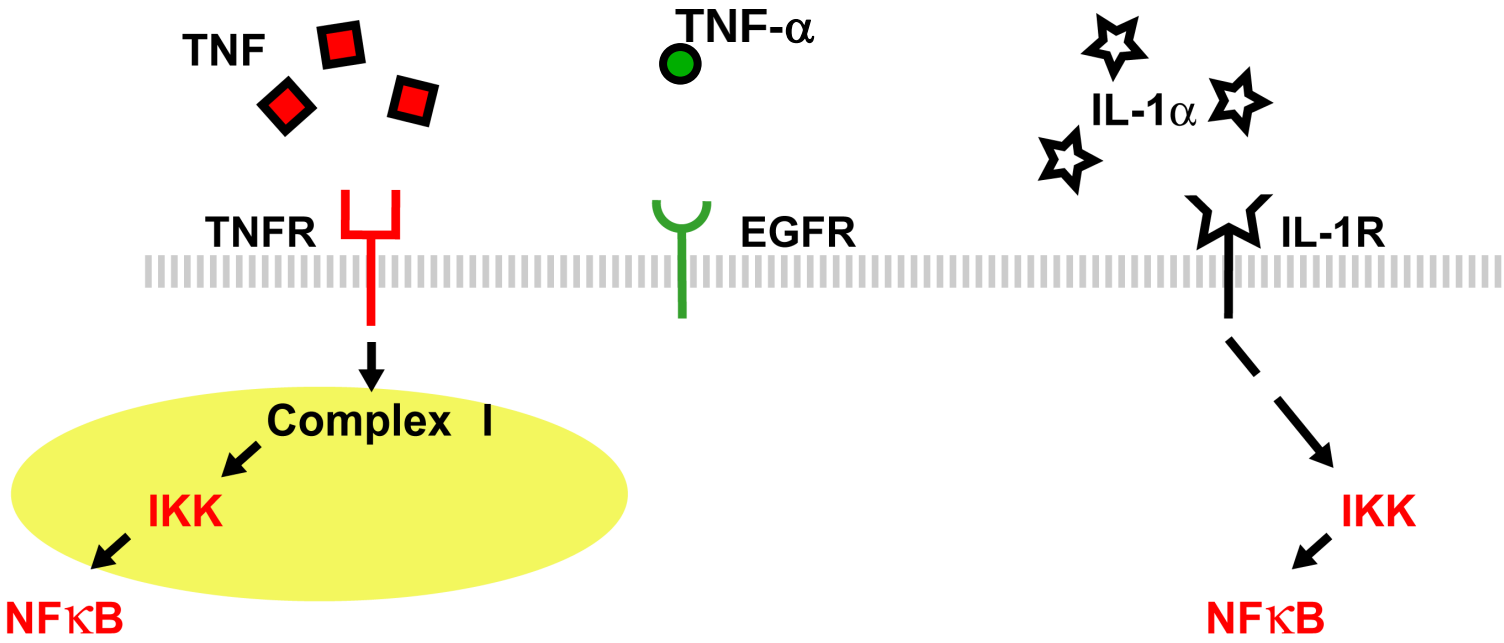


Results: multi-step autocrine crosstalk initiation by TNF



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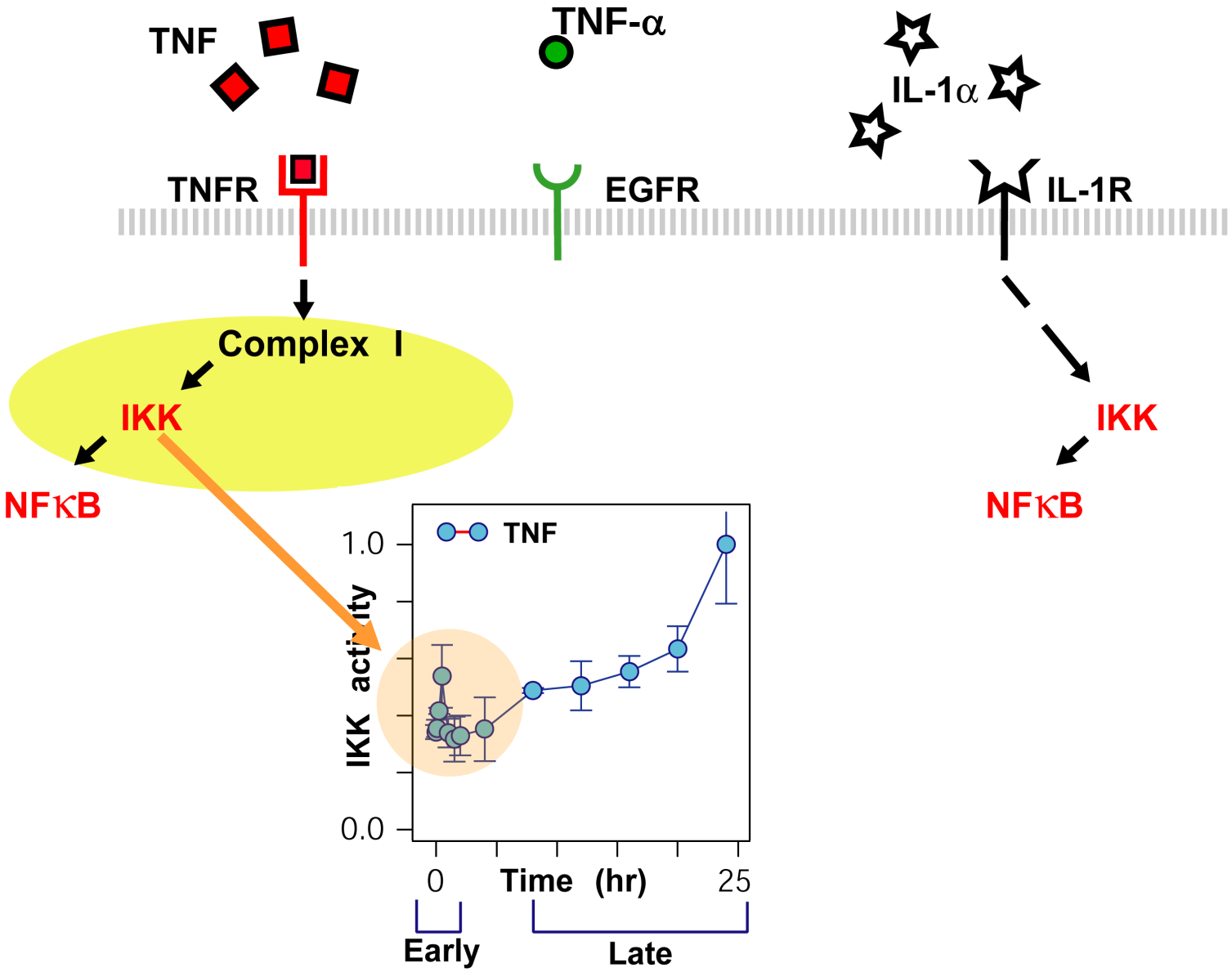

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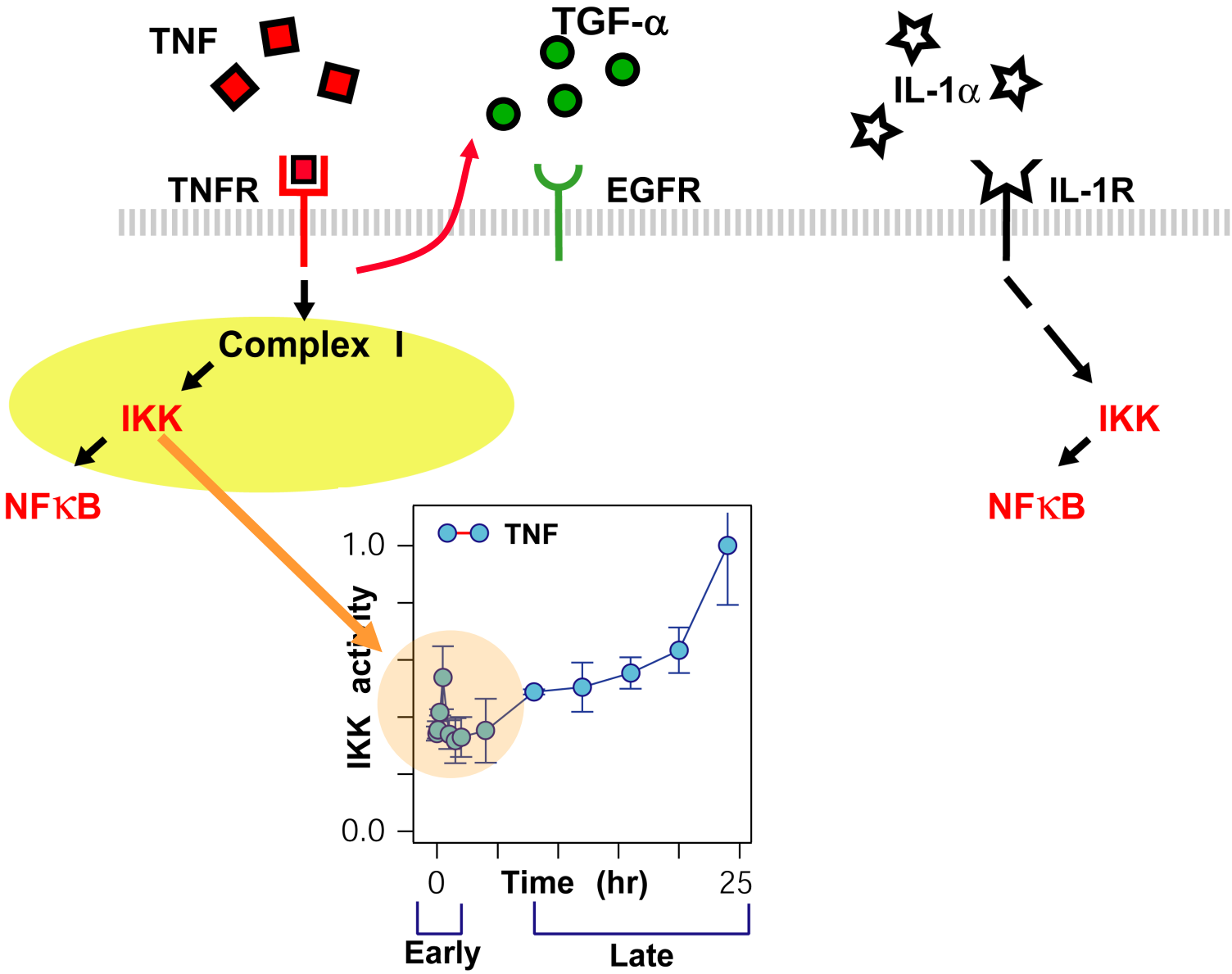

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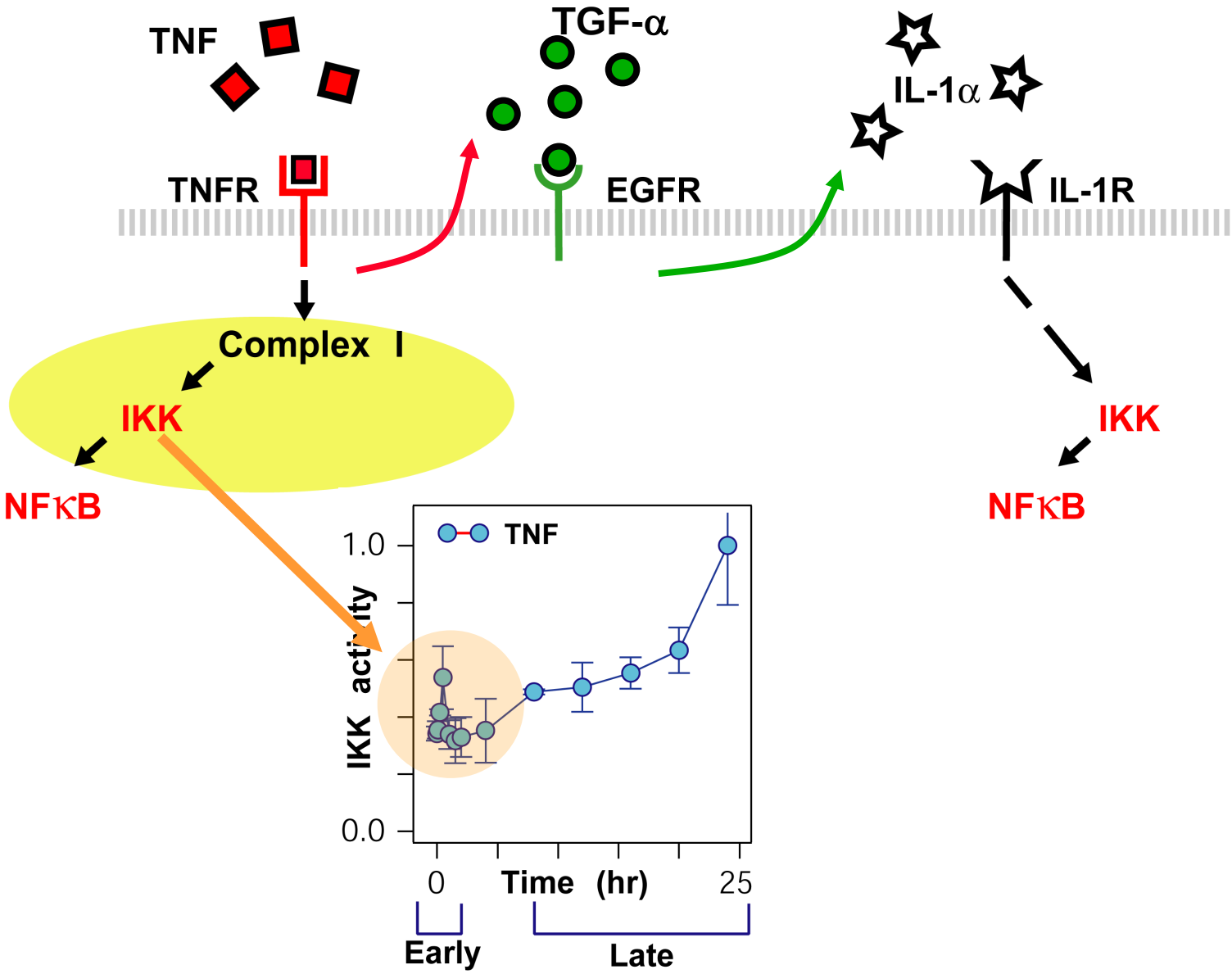

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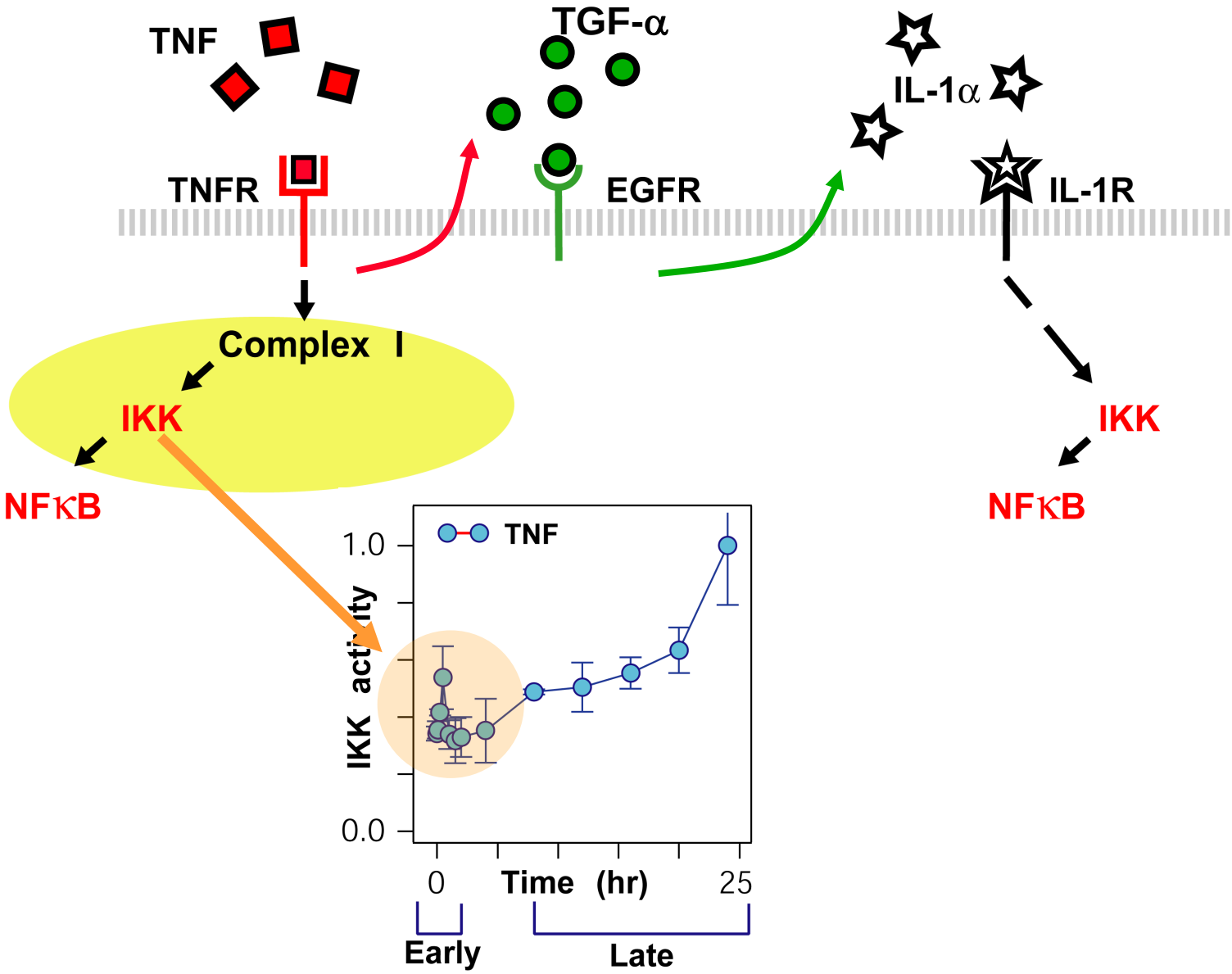

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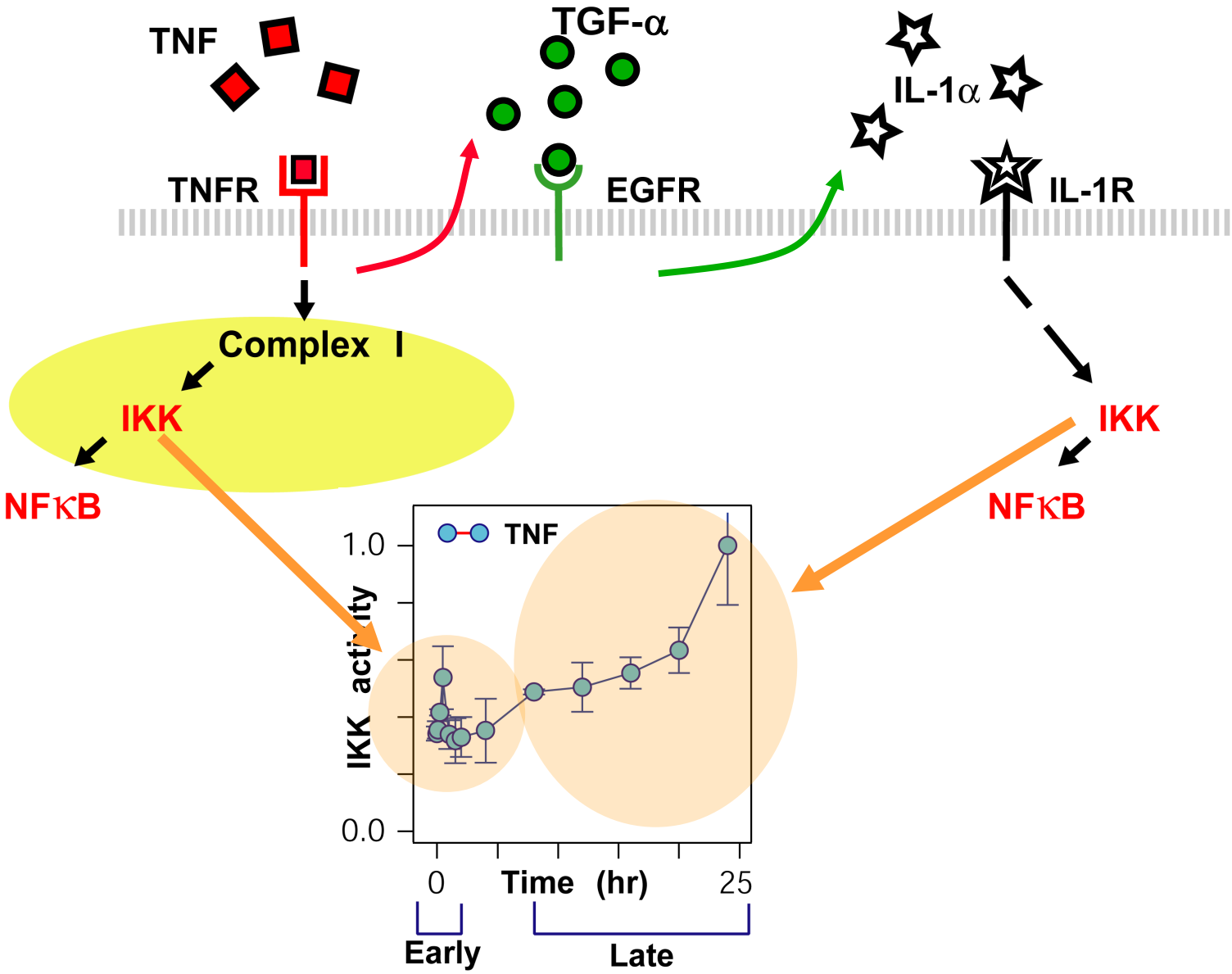

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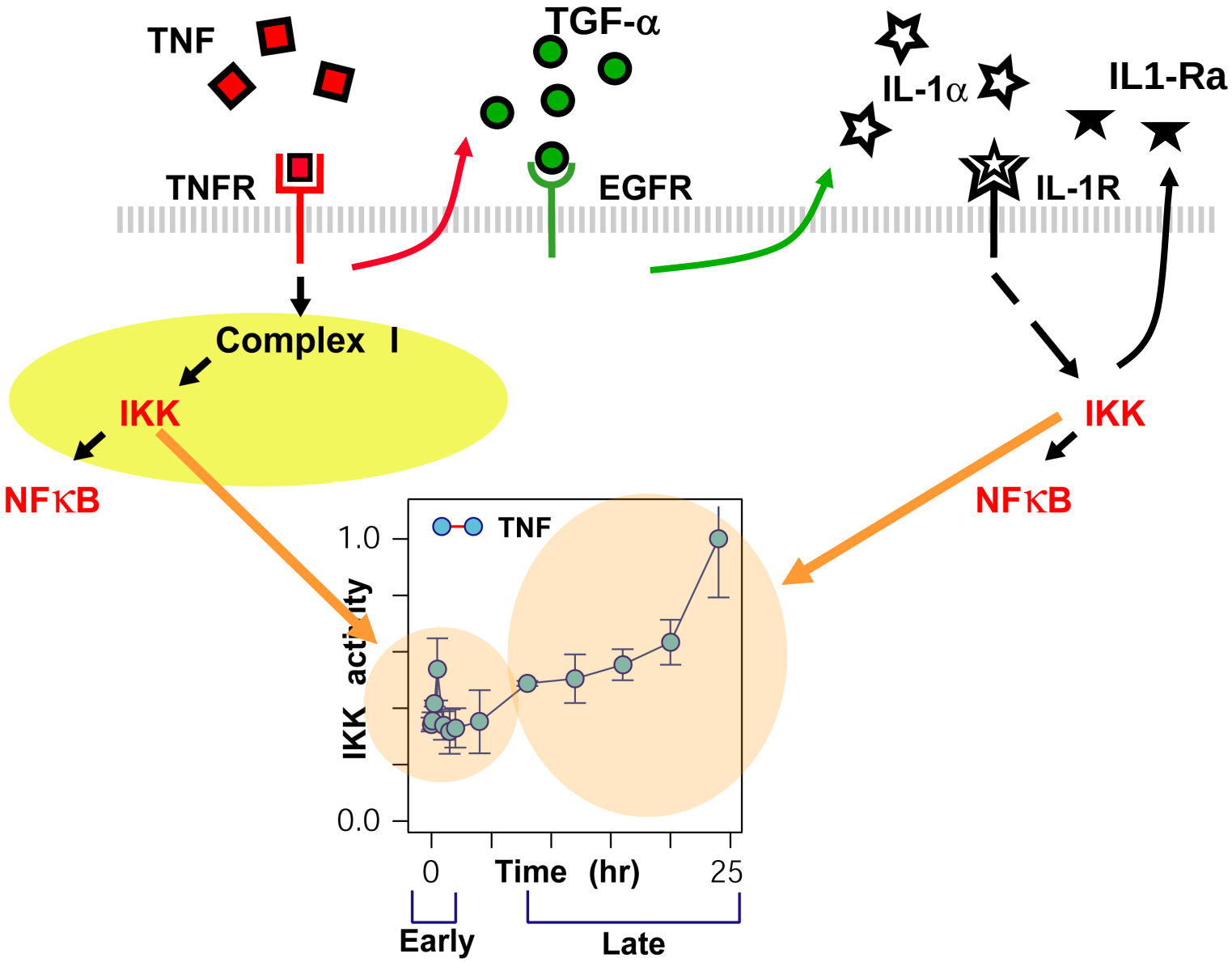

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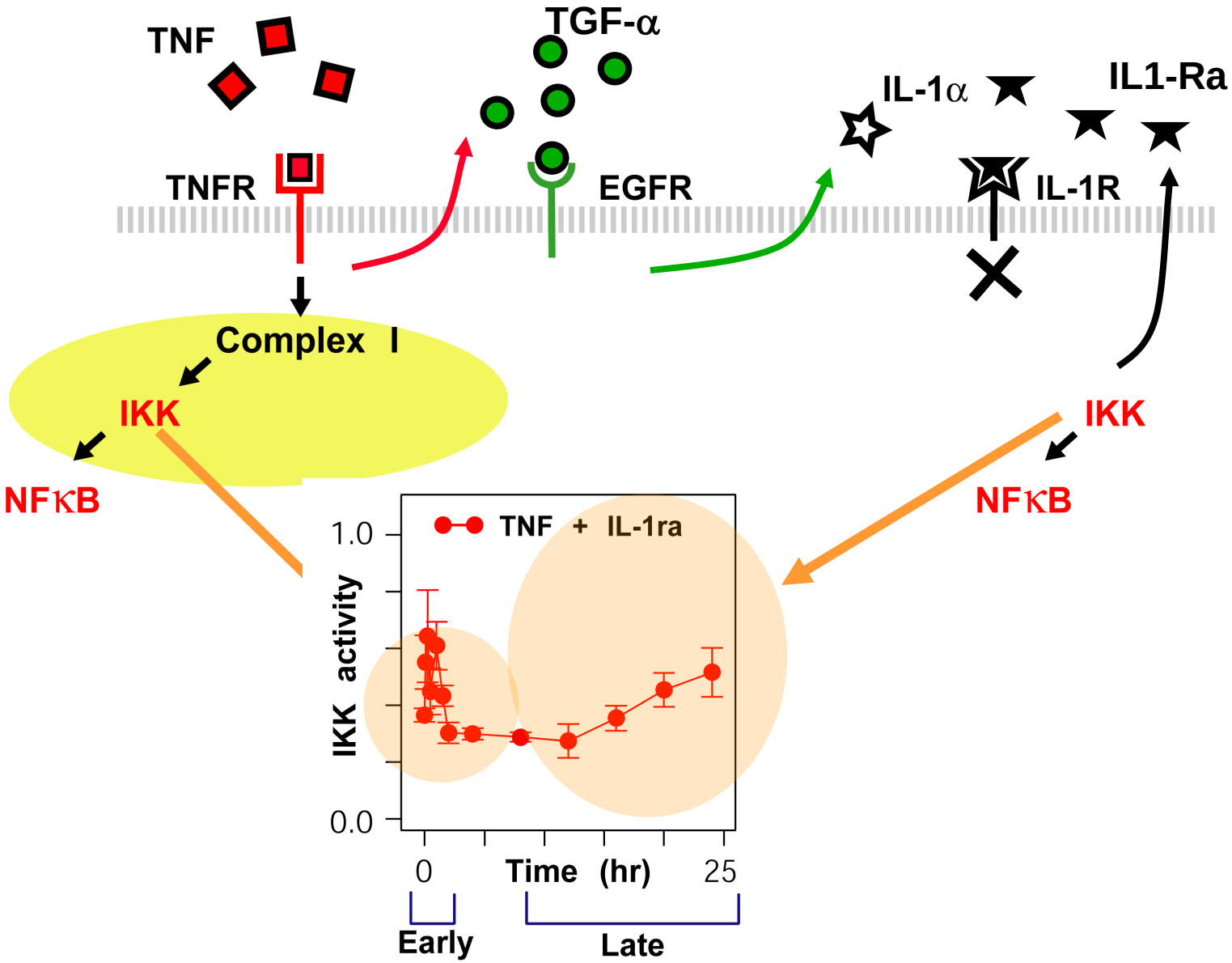

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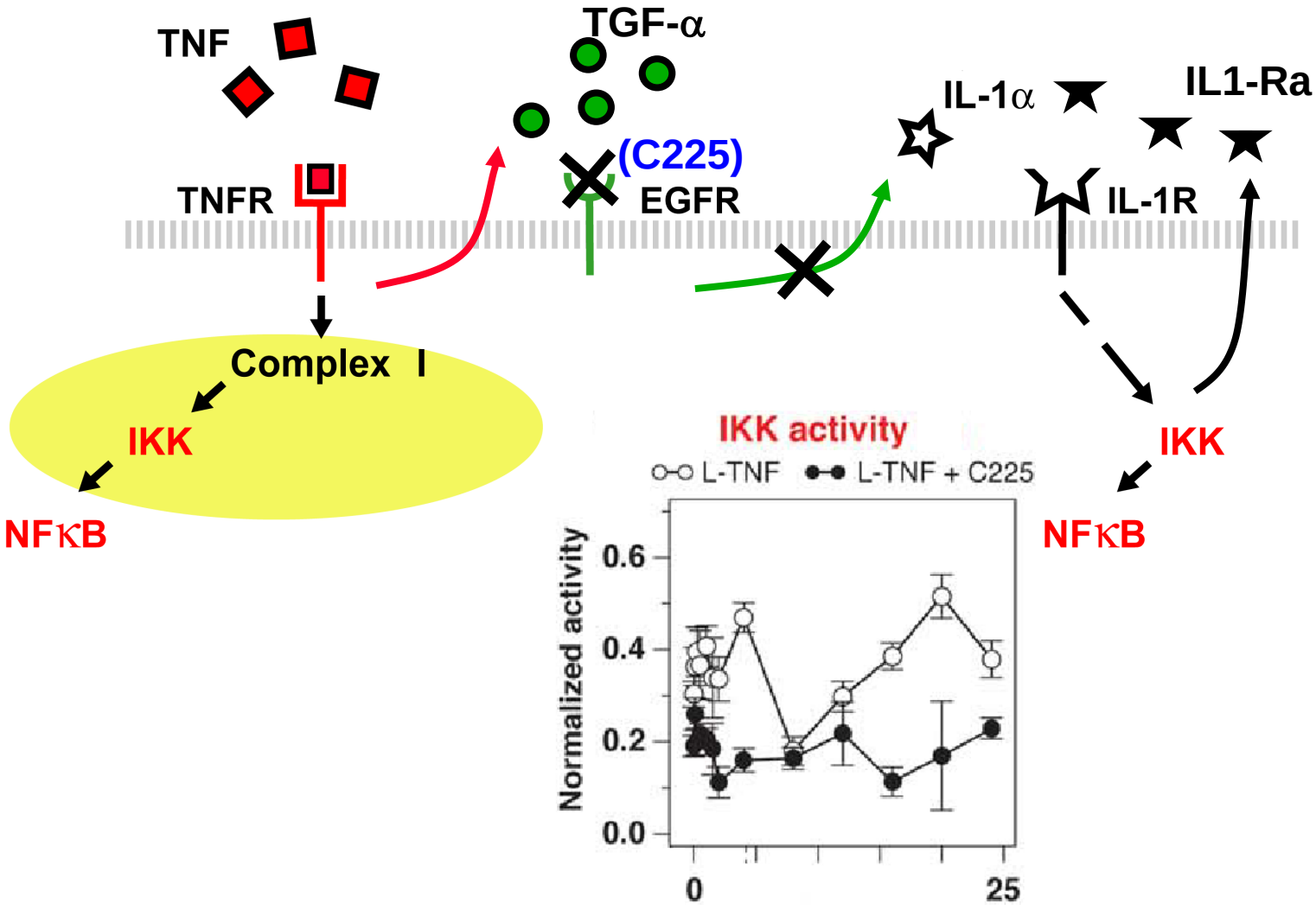

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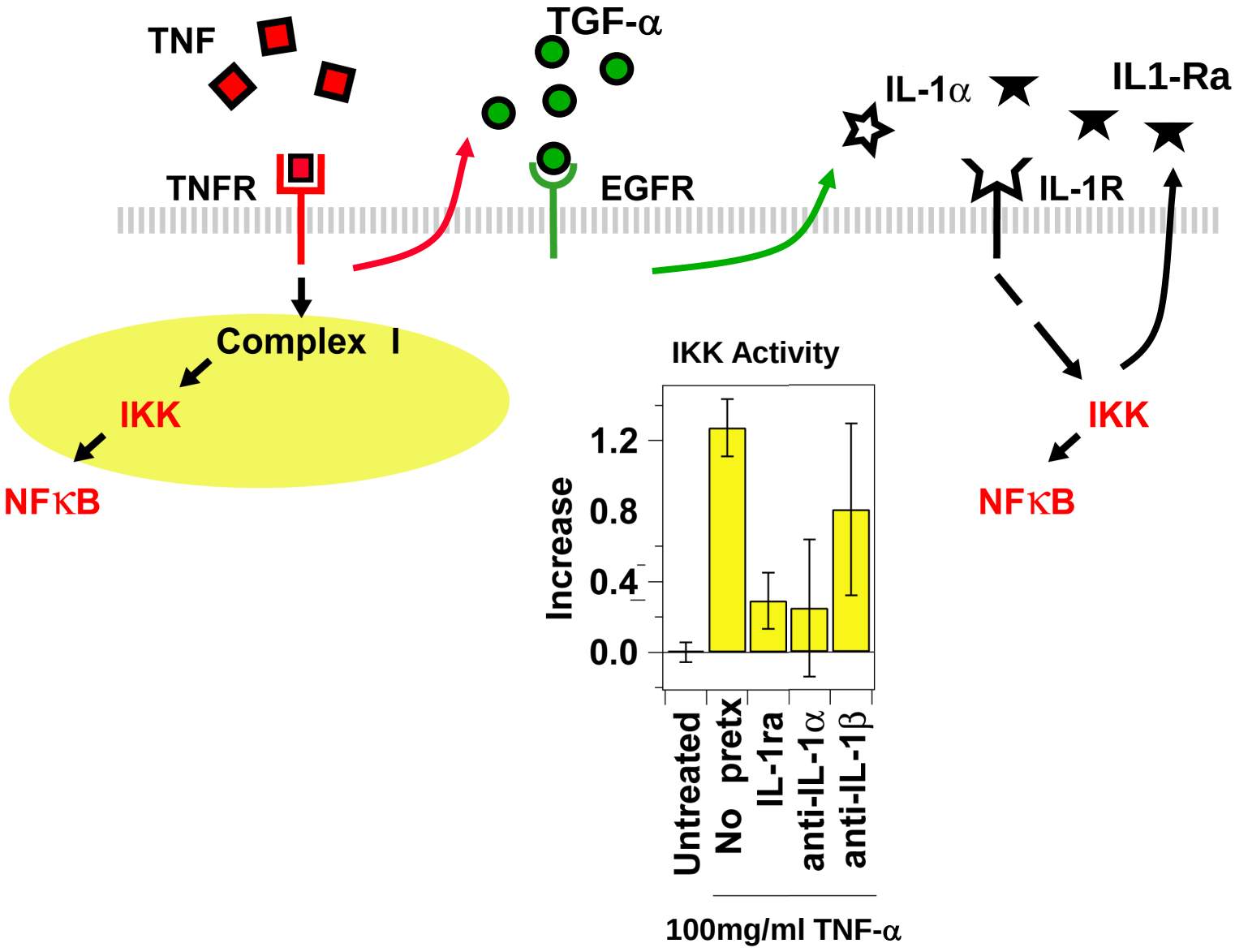

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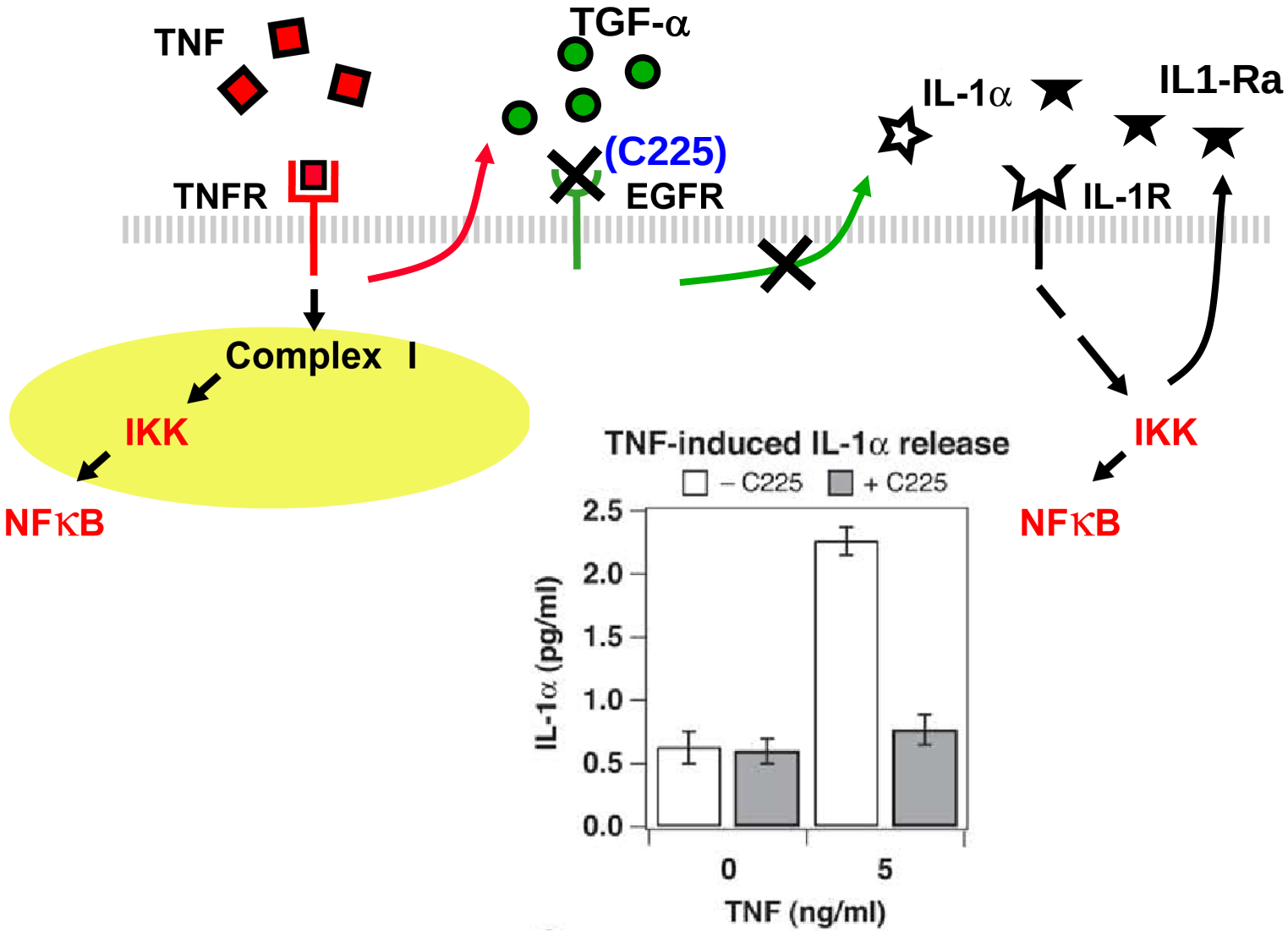
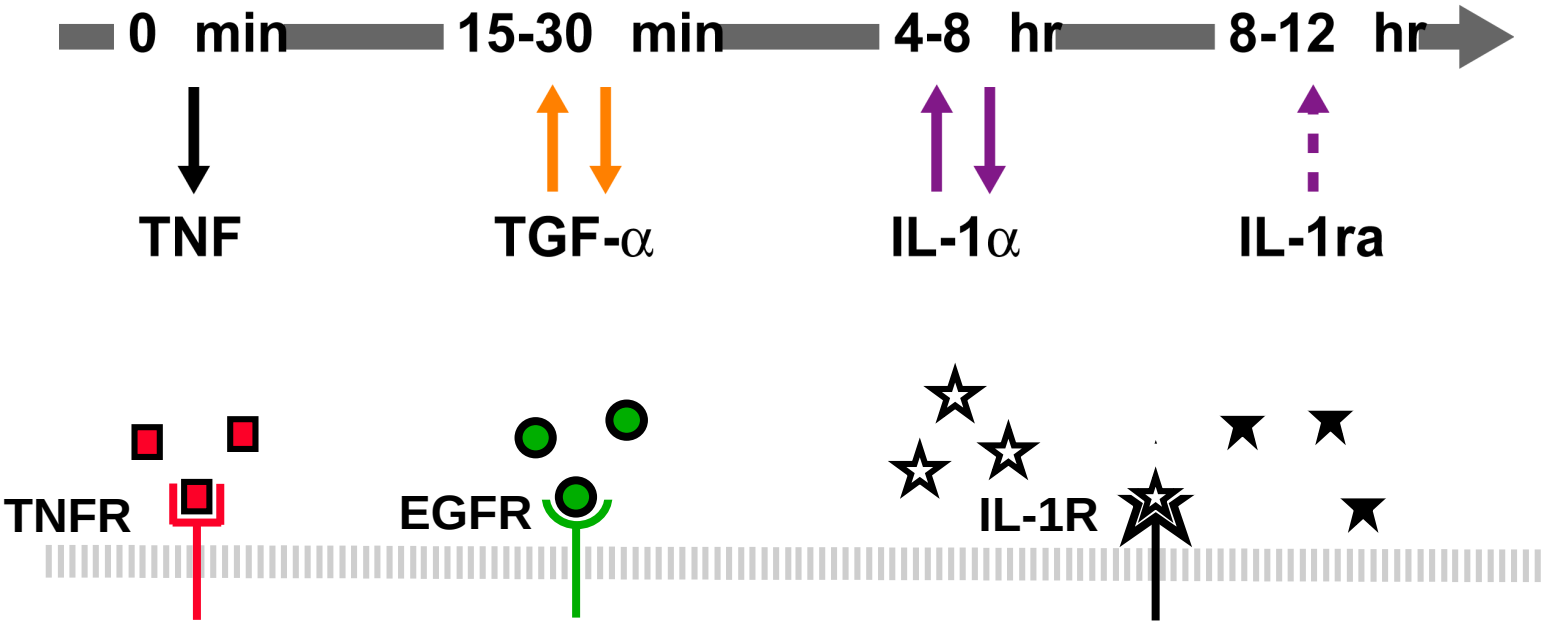
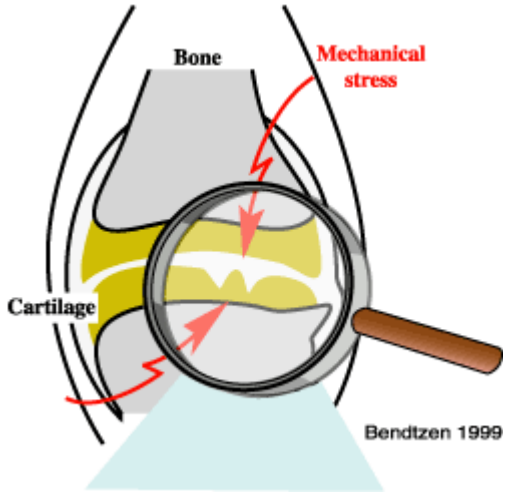
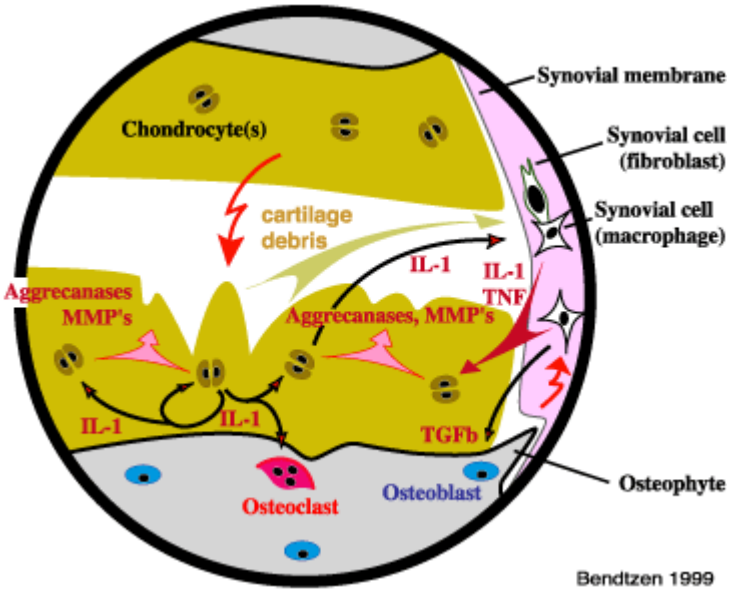


Results: Interactions are time-dependent



The role of IL-1 in cartilage homeostasis

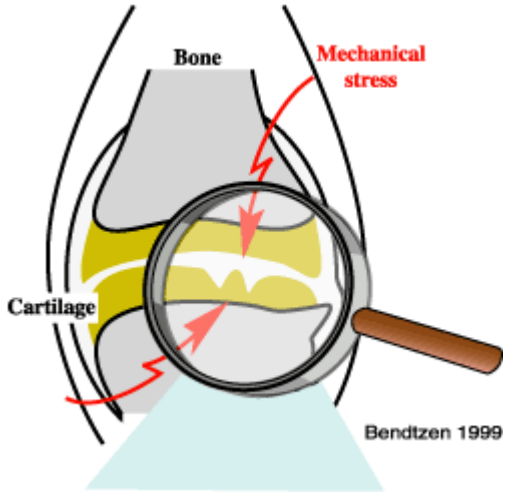


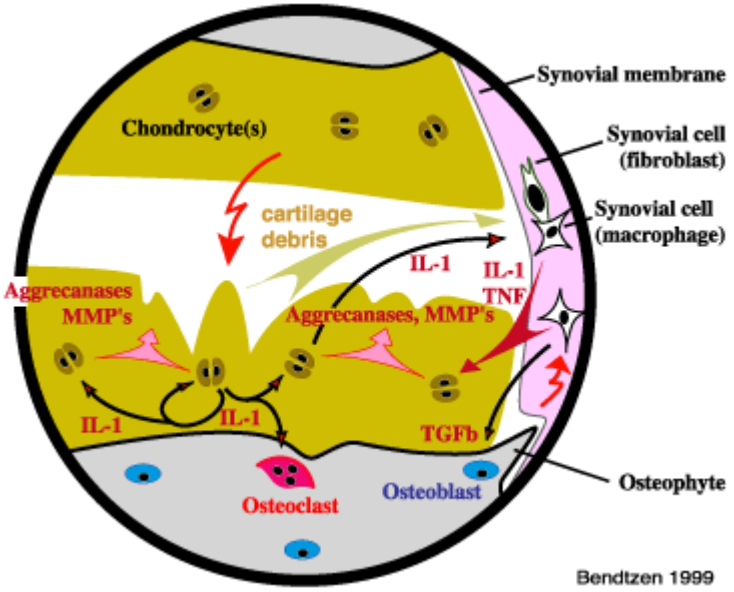


IL-1 in articular cartilage:

- **Originated from macrophages and chondrocytes**
- **Potent catabolic cytokine**
  - Stimulates production of MMPs and other proinflammatory and catabolic genes
- **Implicated in the pathogenesis of cartilage matrix degradation in OA and RA**
  - Detected in arthritic synovial fluid
- **Therapeutic intervention for RA involves blocking the IL-1 pathway with the IL-1Ra antagonist (Anakinra)**

The role of TNF in cartilage homeostasis





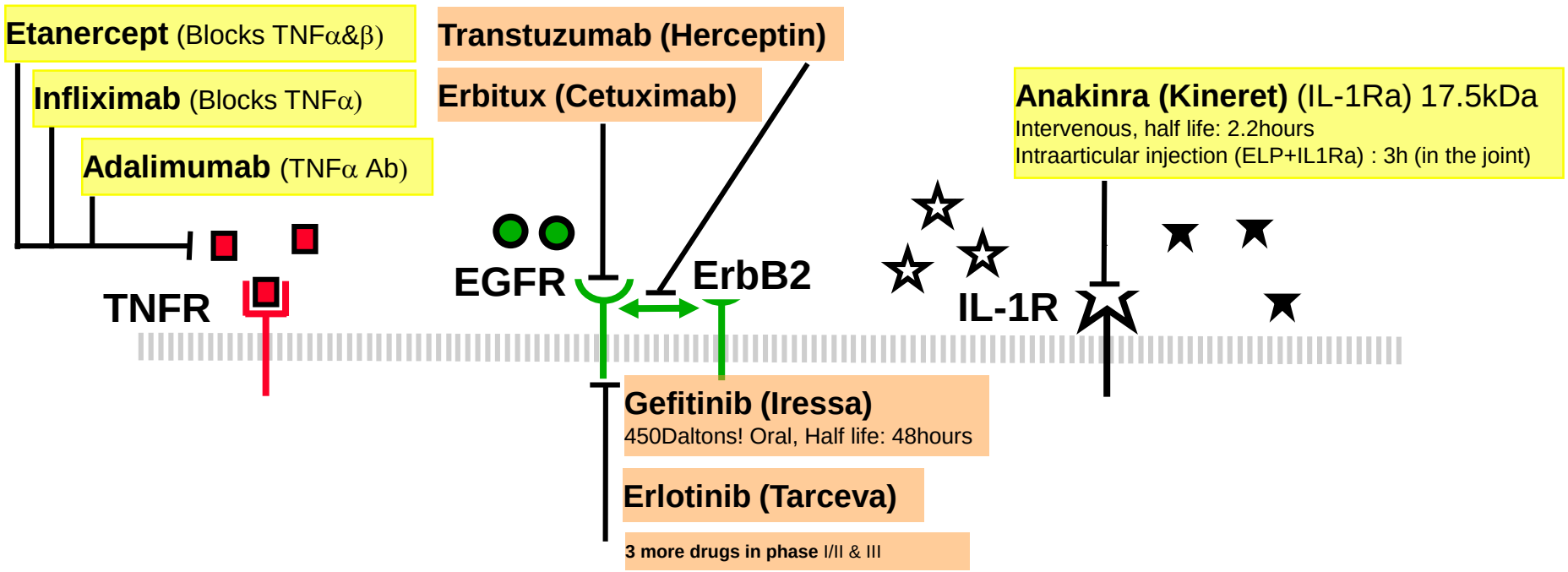
TNF-α in articular cartilage:

- **Effects are similar to those of IL-1**
- **100-fold to 1000-fold less potent on a molar basis than IL-1**
- **VERY POTENT synergistic effects w/ IL-1**
  - Simultaneous injection of IL-1 and TNF-a in articular cartilage far exceeds the catabolic effects observed with injection of either cytokine alone
- **May act via the NFκB transcriptional factor**
- **Etanercept (Enbrel), Infliximab (Remicade) and Adalimumab (Humira) are TNFα blocking agents that are been used in RA to block the effects of TNF-*a***
- ***Certain serious, but uncommon, adverse events have been observed with all three TNFalpha blocking agents, including serious bacterial infections, tuberculosis (TB) and certain opportunistic infections, and lupus-like reactions. Blockade of interleukin-1 activity with Anakinra appears to be relatively safe*** .

Therapeutic interventions

DRUGS for Arthritis

Cancer Drugs



Hypotheses

- **TNFα initiates a multi step autocrine cross talk that stimulates the production of IL-1α via the EGFR pathway**

- **Disruption EGFR pathway with small inhibitors can downregulate the TNF induced IL-1**

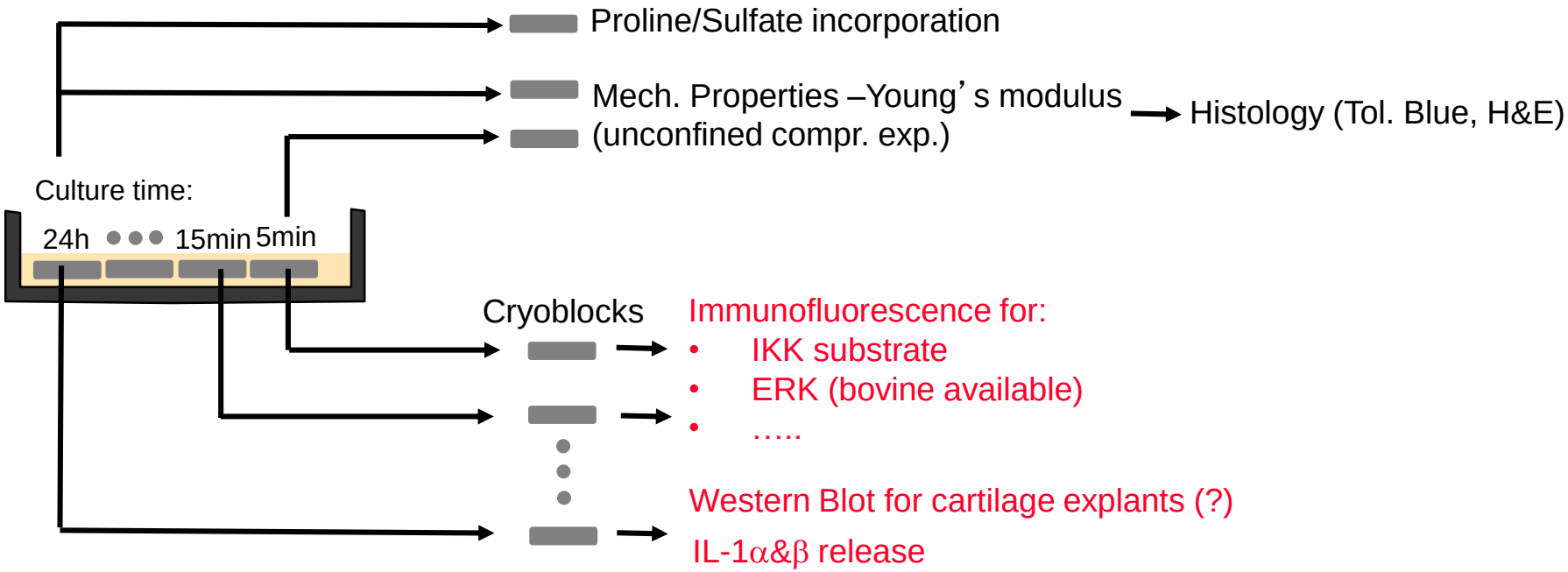
Materials and Methods

Human(?) Normal Cartilage

Culture w/ 10% Serum + TNFa (0, 5 & 100 ng/ml)

- With or without EGFR inhibitor (preferably Iressa that diffuses through the cartilage)
- With or without IL1-Ra

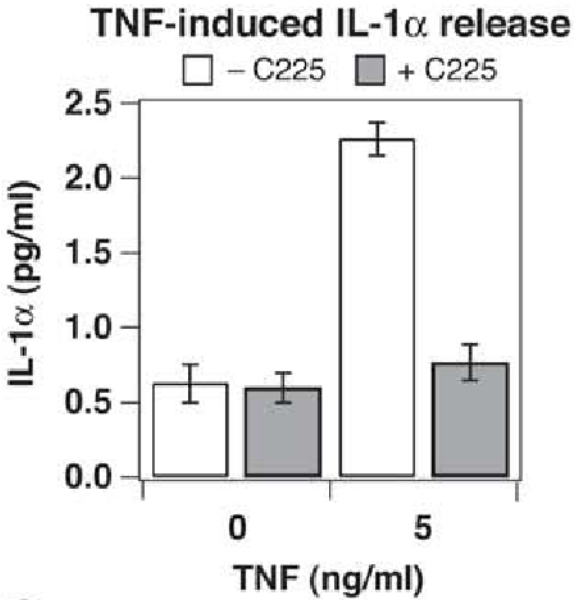
Experimental Setup



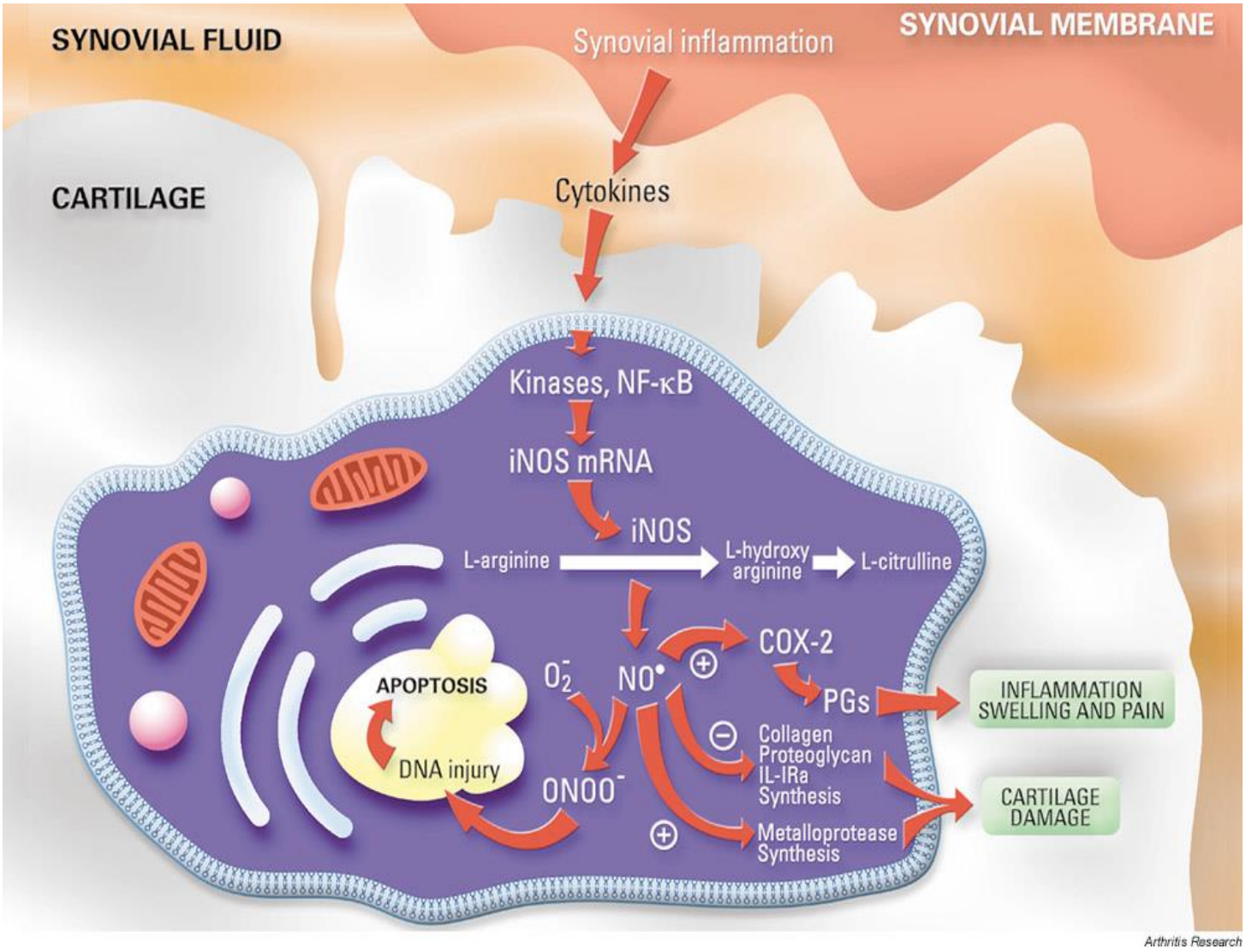
Goal

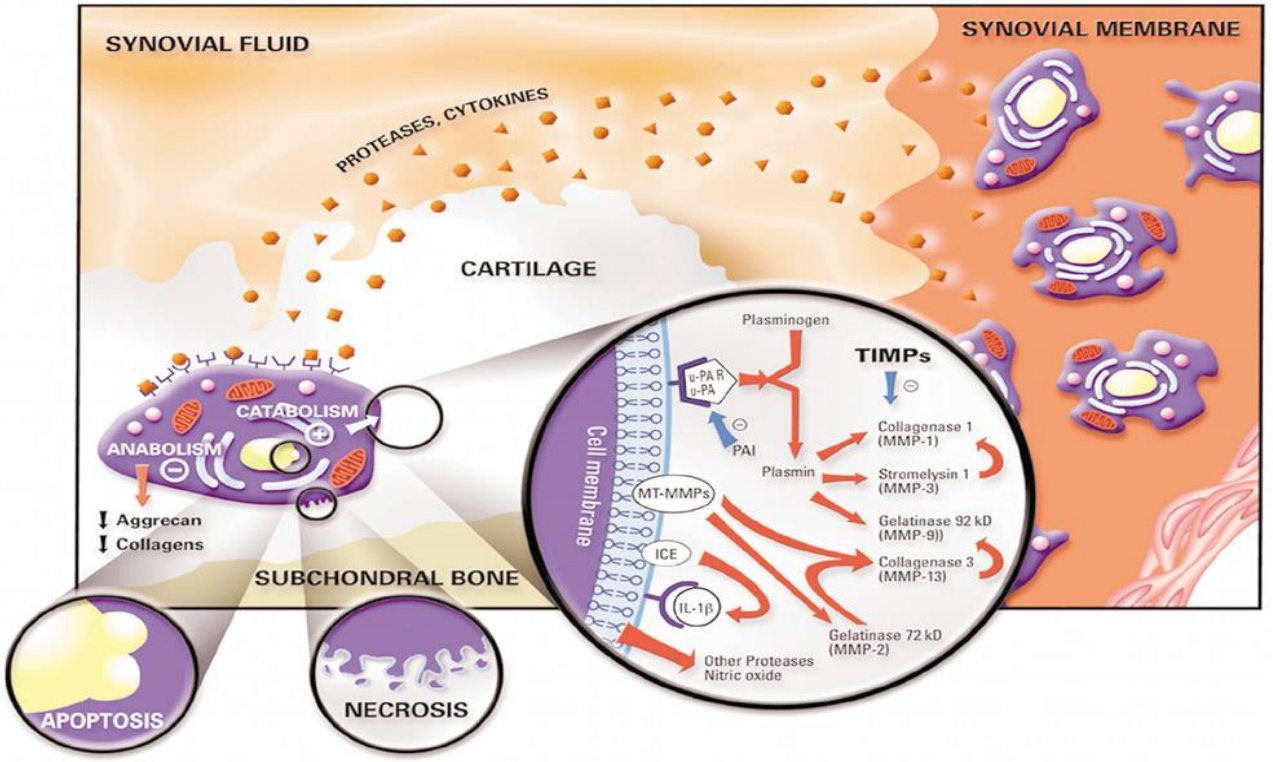
- Disruption of the EGFR pathway may inhibit the TNF+IL-1 synergistic effect

- If IL-1 comes mostly from the TNF induced EGFR pathway, tyrosine kinase inhibitors can be proved a valuable therapeutic target for RA
  - Iressa has significantly higher half life than IL-1Ra (48h vs. 2.2 hours)
  - Iressa is significantly smaller than IL-1Ra and can easily diffuse through cartilage



Thank You


SYNOVIAL FLUID
Synovial inflammation
SYNOVIAL MEMBRANE
CARTILAGE
Cytokines
Kinases, NF-κB
iNOS mRNA
iNOS
L-arginine
L-hydroxy arginine
L-citrulline
COX-2
PGs
INFLAMMATION SWELLING AND PAIN
APOPTOSIS
DNA injury
O_2^-
NO•
ONOO⁻
Collagen
Proteoglycan
IL-IRa
Synthesis
Metalloprotease
Synthesis
CARTILAGE DAMAGE
Arthritis Research



Arthritis Research

Cartilage tissue engineering in alginate-based scaffolds

Leonidas G. Alexopoulos

Cartilage tissue engineering

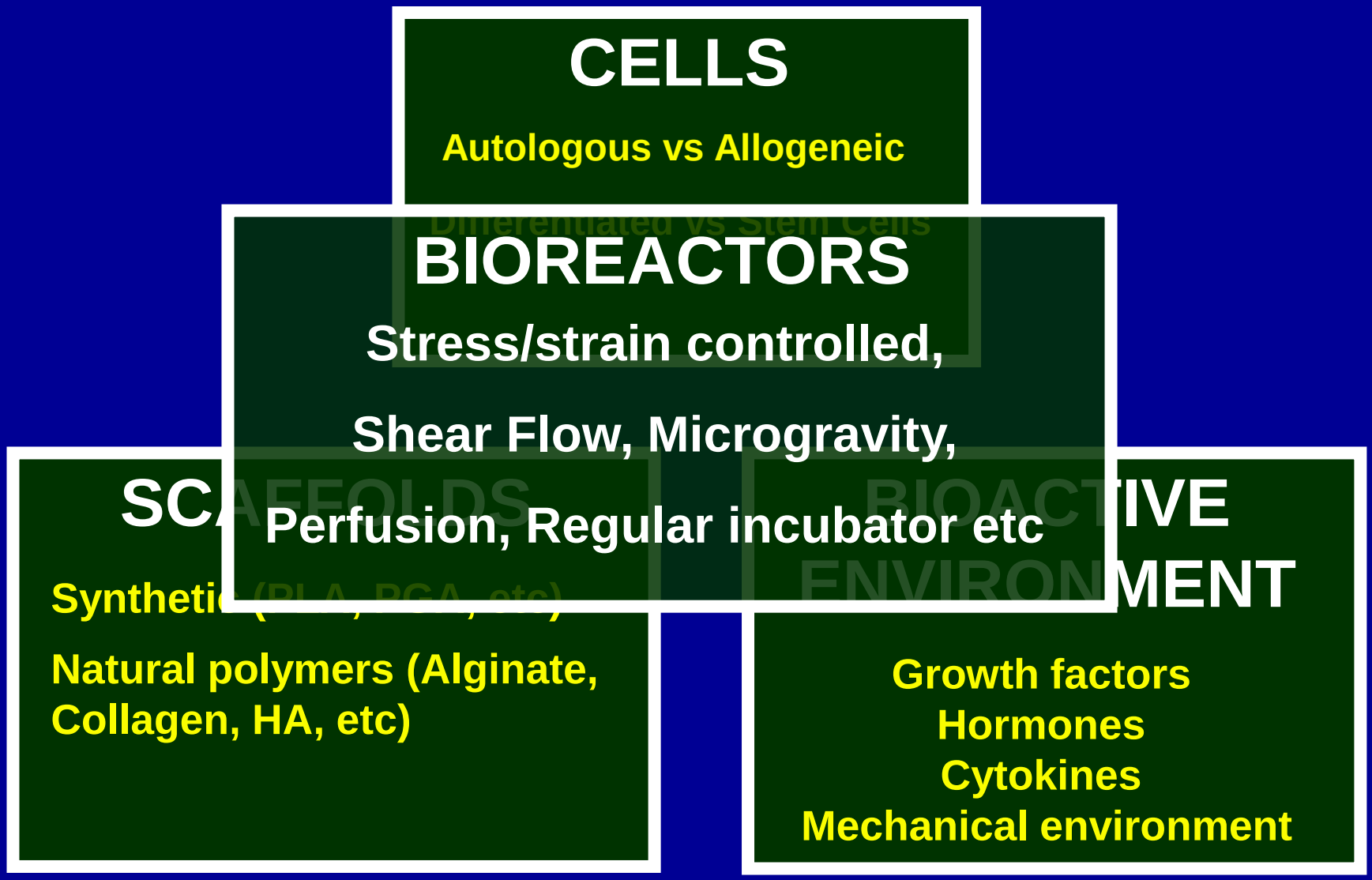
The combination of living cells, scaffolds, and a biologically active environment to replace or repair injured cartilage

GOALS:

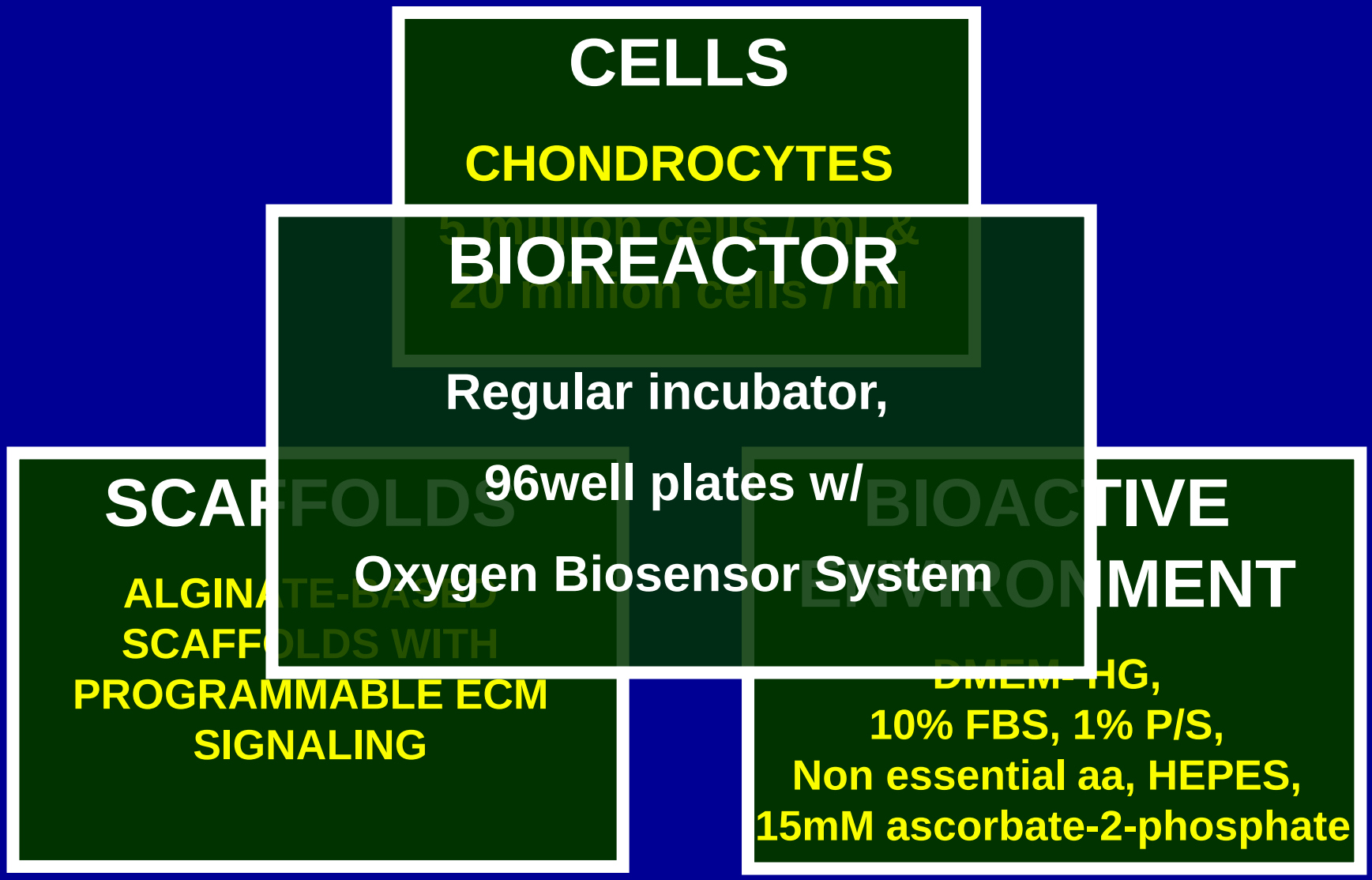
- **Restore the biomechanical function of the joint**
- **Restore the biological activity of the cartilage**

Butler et al (2000) JBE p.570-574, Guilak et al (2001) Clinical Orthopaedics and Related Research 391S, p.S295-S305

Tissue engineering

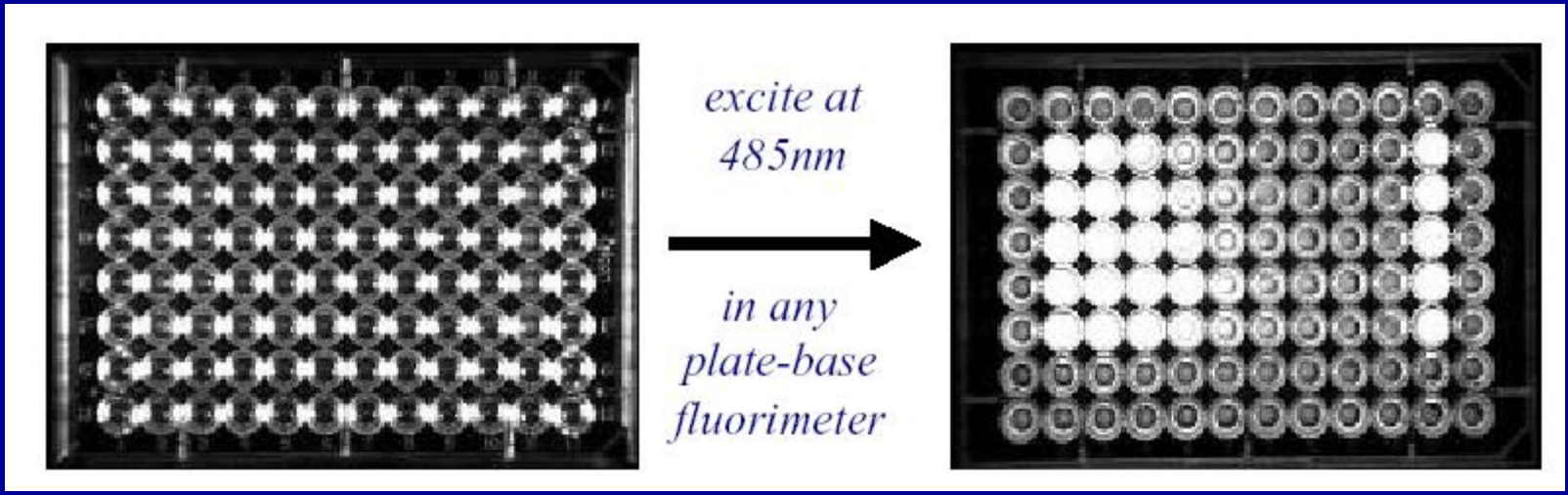


Materials and Methods



BD Oxygen Biosensor System (OBS)

- 96 well plate
- Oxygen-sensitive fluorophore embedded into a silicon rubber matrix have been applied to the bottom of the chamber
- Readings are performed in a regular plate reader



Alginate-based Scaffolds

- 2mm x 5mm disks were made 2% Alginate
- 3 different molecules were embedded into the disks:
  - Collagen II
  - Collagen VI
  - Fibronectin
  - No modification

Hypotheses

Modified alginate scaffolds can provide an anabolic environment for cartilage tissue engineering

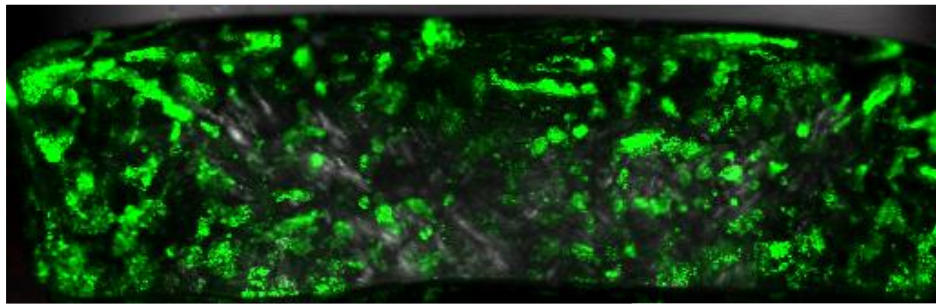
&

OBS plates can provide a non-invasive and high throughput method to monitor tissue growth

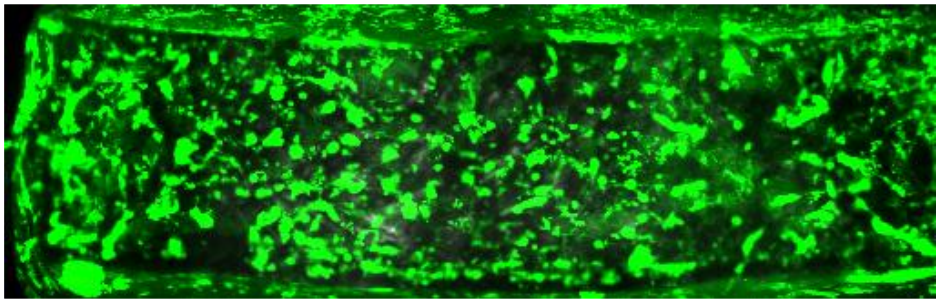
Live Dead Assay

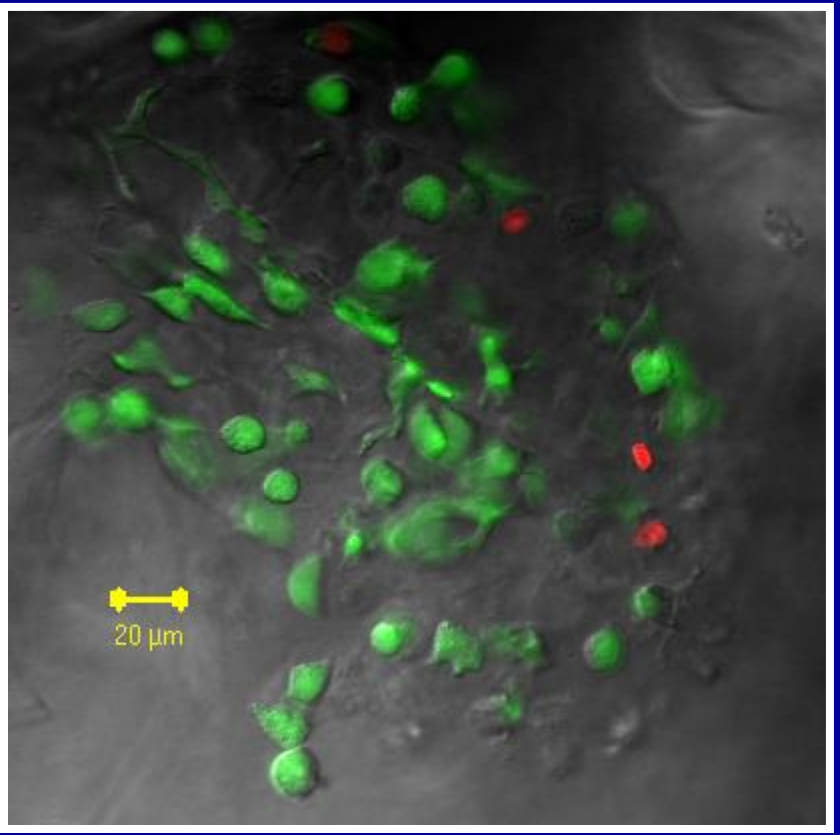
Collagen type II scaffold – day 14

50,ooo cells/scaffold

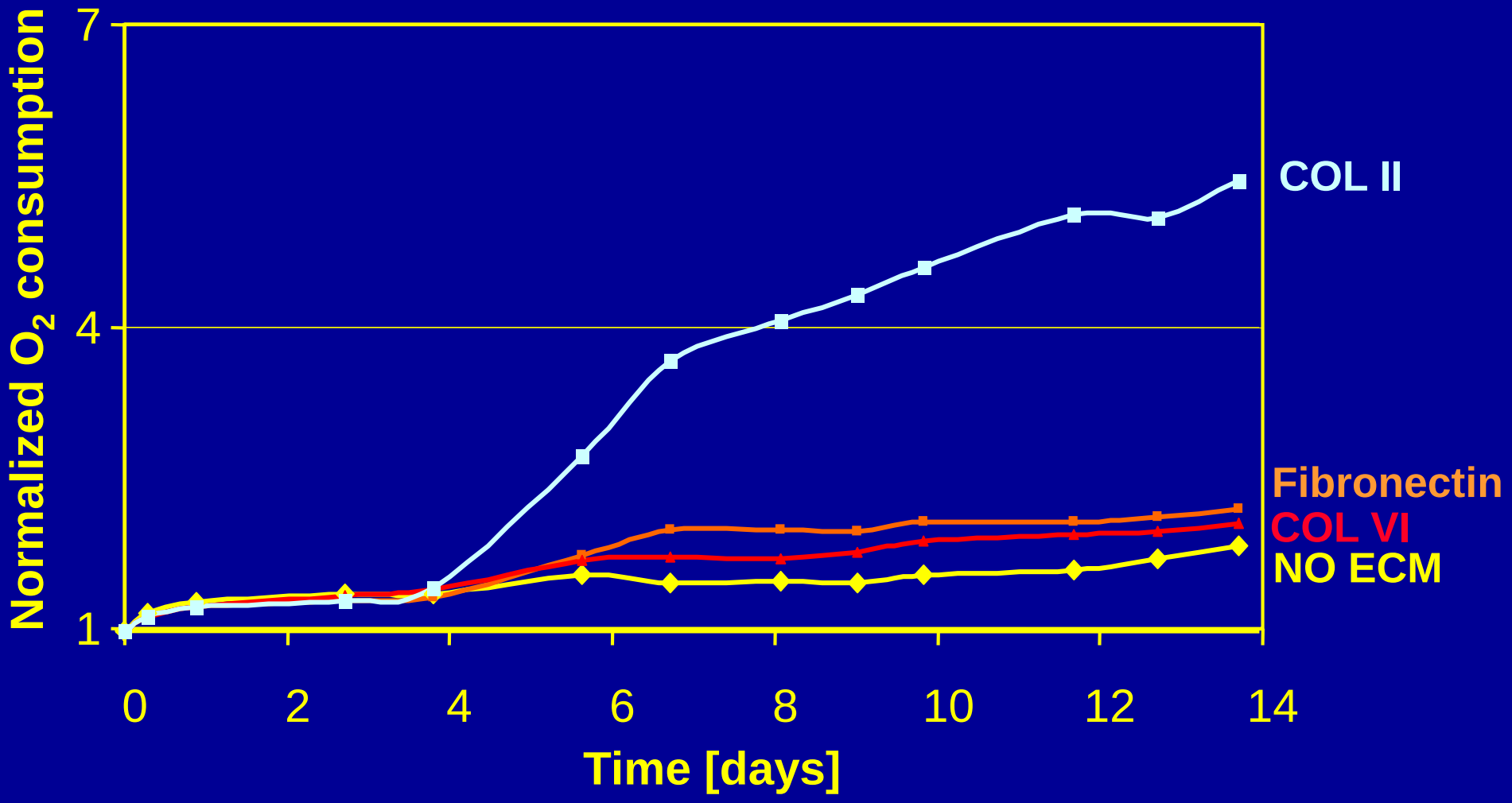


250,ooo cells/scaffold



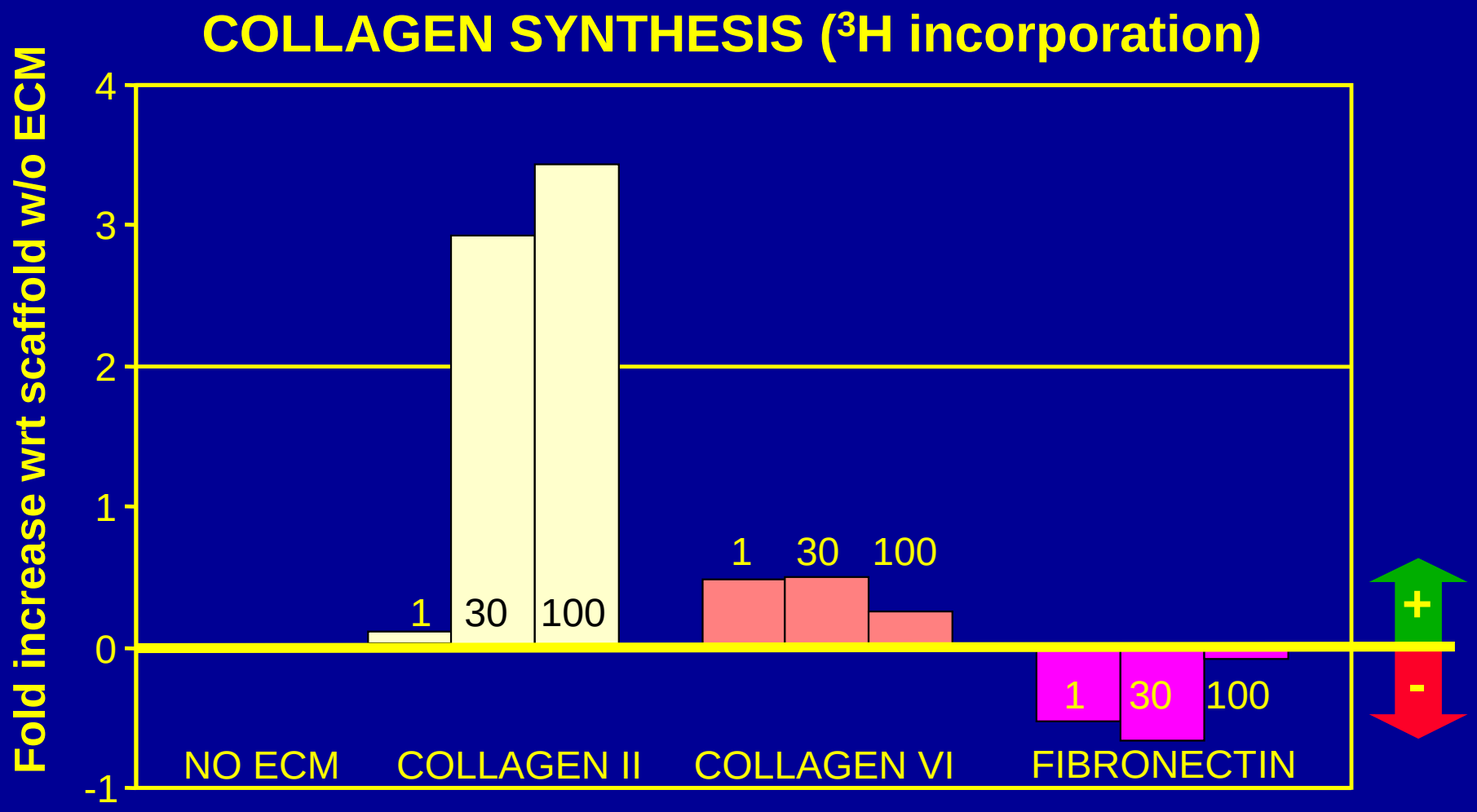


Collagen II has anabolic effects



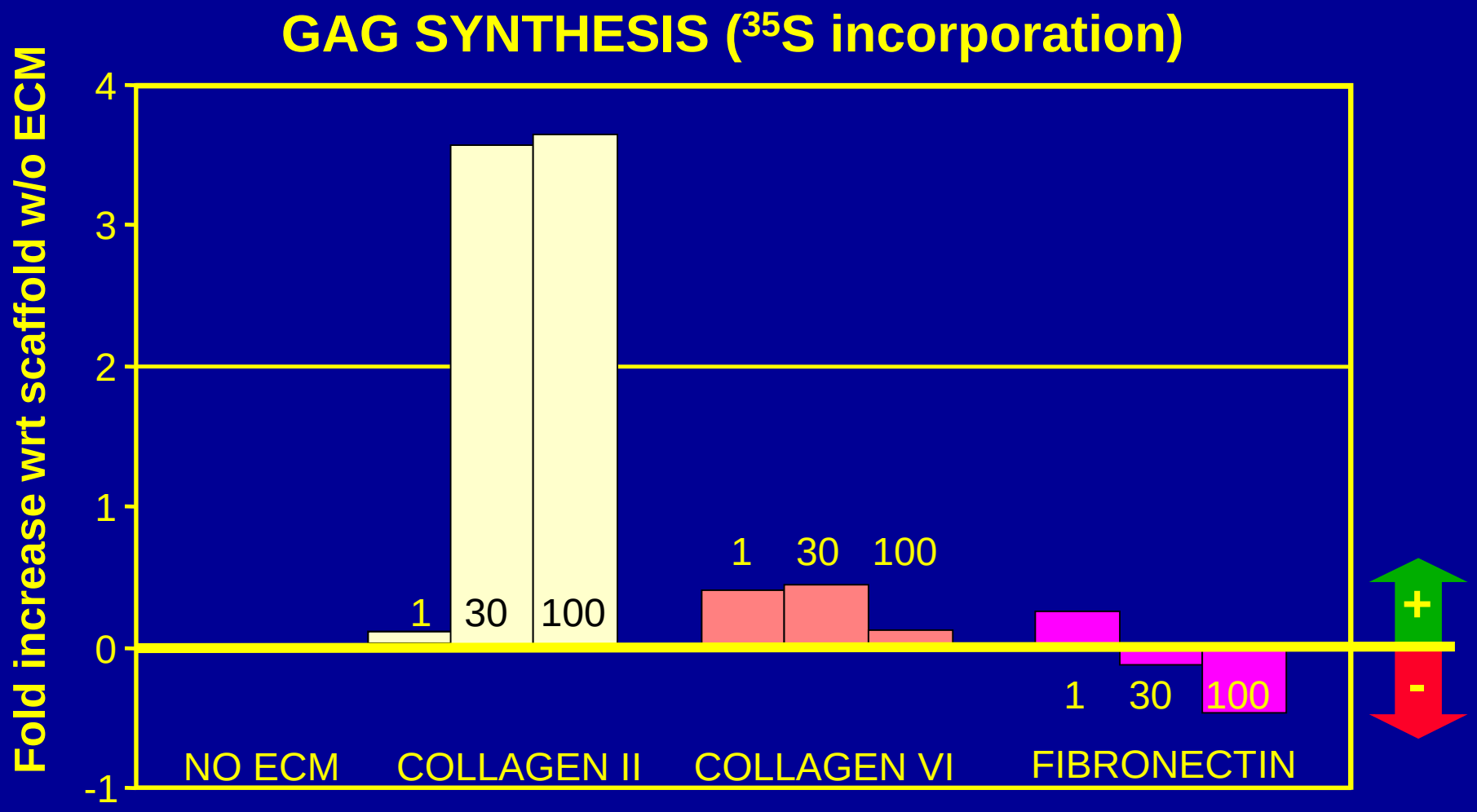
250,ooo cells / scaffold – ECM concentration: 100mg/ml

Collagen II has anabolic effects



250,ooo cells / scaffold. Concentration is in [mg/ml]

Collagen II has anabolic effects



250,ooo cells / scaffold. Concentration is in [mg/ml]

Work in progress

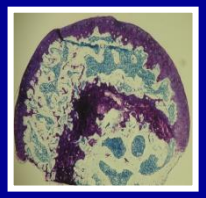
- Total DNA (picogreen assay) and cell counting on dissolved scaffolds will reveal proliferation activity
- Total Collagen (Hydroxyproline assay) and total GAG (DMB assay) will reveal the metabolic activity of the scaffolds
- Histology and immunohistochemistry will determine the quality of the metabolic activity (collagen II vs collagen I)
- Mechanical tests can reveal the biomechanical function of the constructs

GENERAL CONCLUSIONS

1. Alginate scaffolds modified with collagen II might provide an anabolic environment for cartilage tissue engineering

2. Oxygen Biosensor plates (OBS) might provide a non-invasive high throughput method to monitor tissue growth

THANK YOU



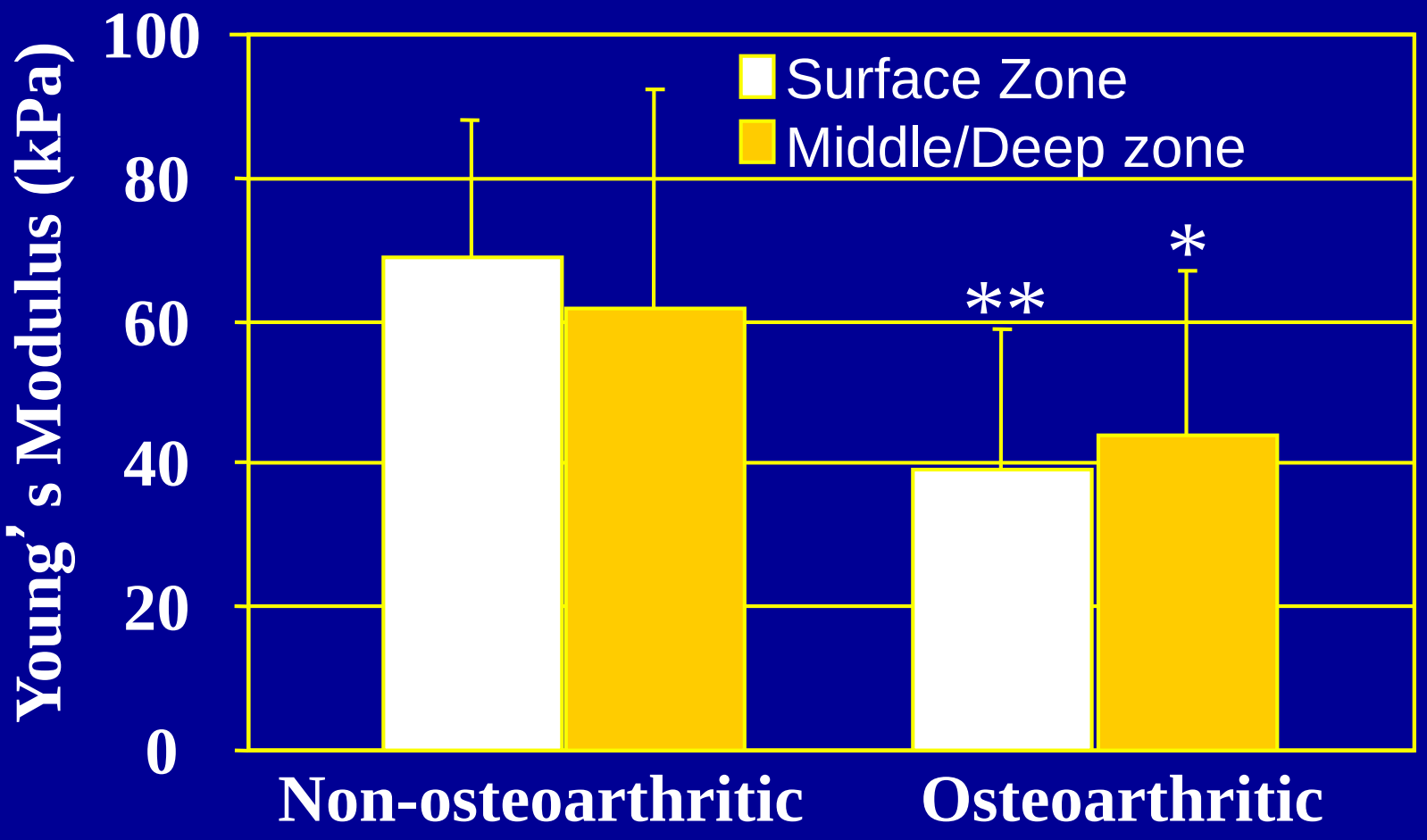
Osteoarthritic grading

	Grade
I. Articular Surface (fibrillation/clefts)	0-4
II. Loss of Staining	0-3
III. Fibrocartilage	0-2
TOTAL SCORE	0 to 9

Carlson et al., 2002, Fink et al. 2002

No zonal differences in the Young's modulus

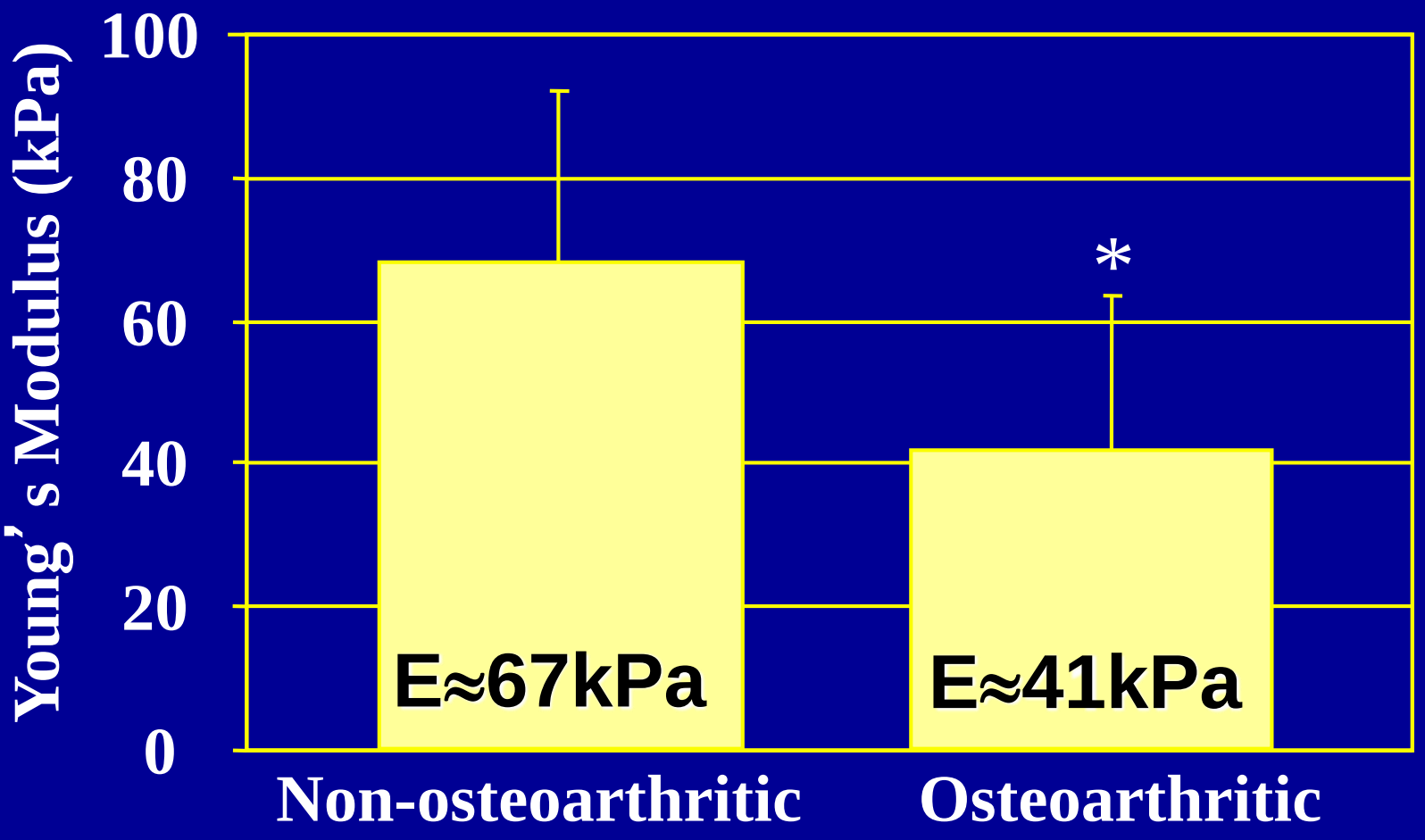
ANALYTICAL MODEL



N>15 per group, *p<0.05, **p<0.001 vs. the non-OA counterpart

Young's modulus is reduced with OA

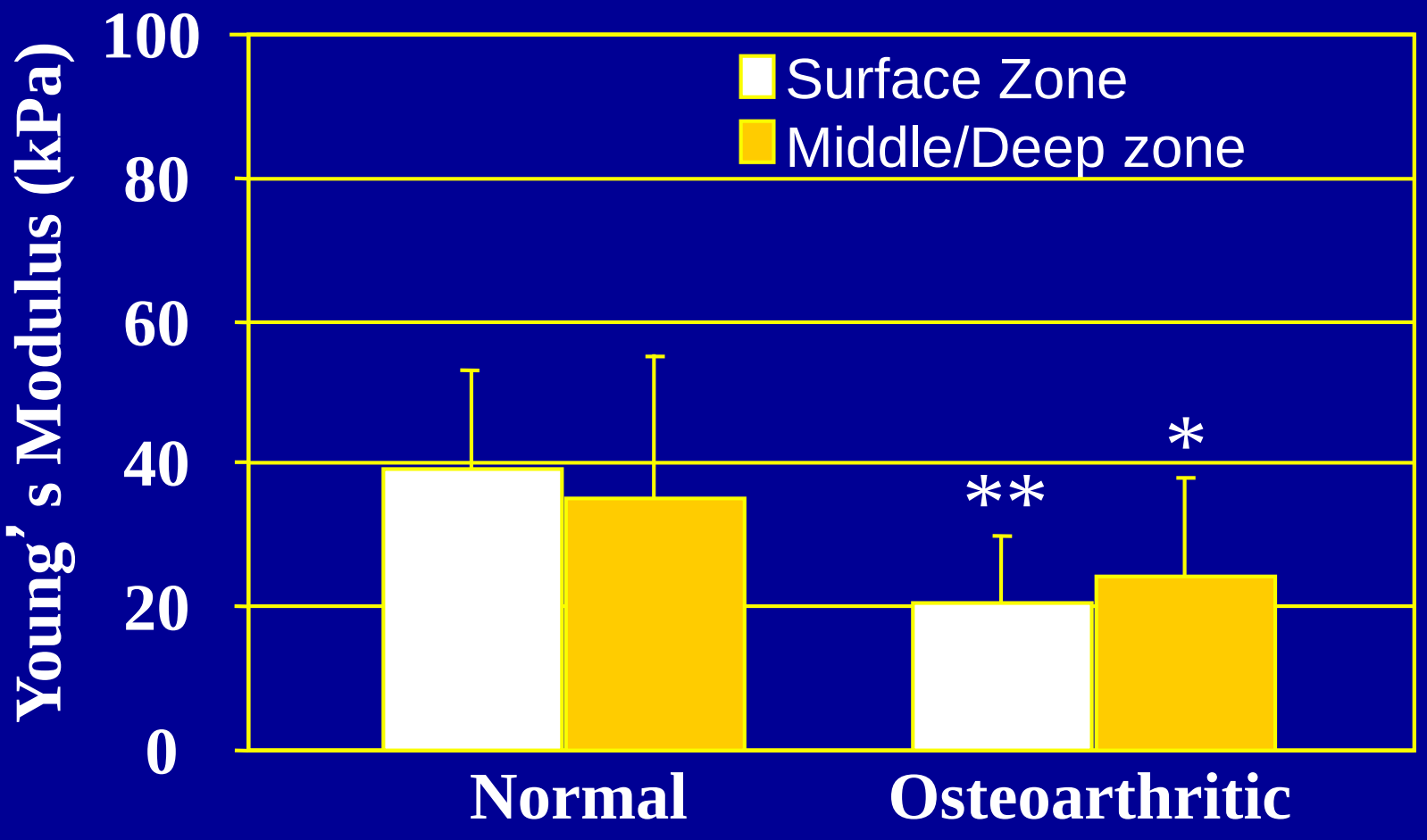
ANALYTICAL MODEL



N>30 per group, *p<0.001 vs. the non-OA controls

No zonal differences in the Young's modulus

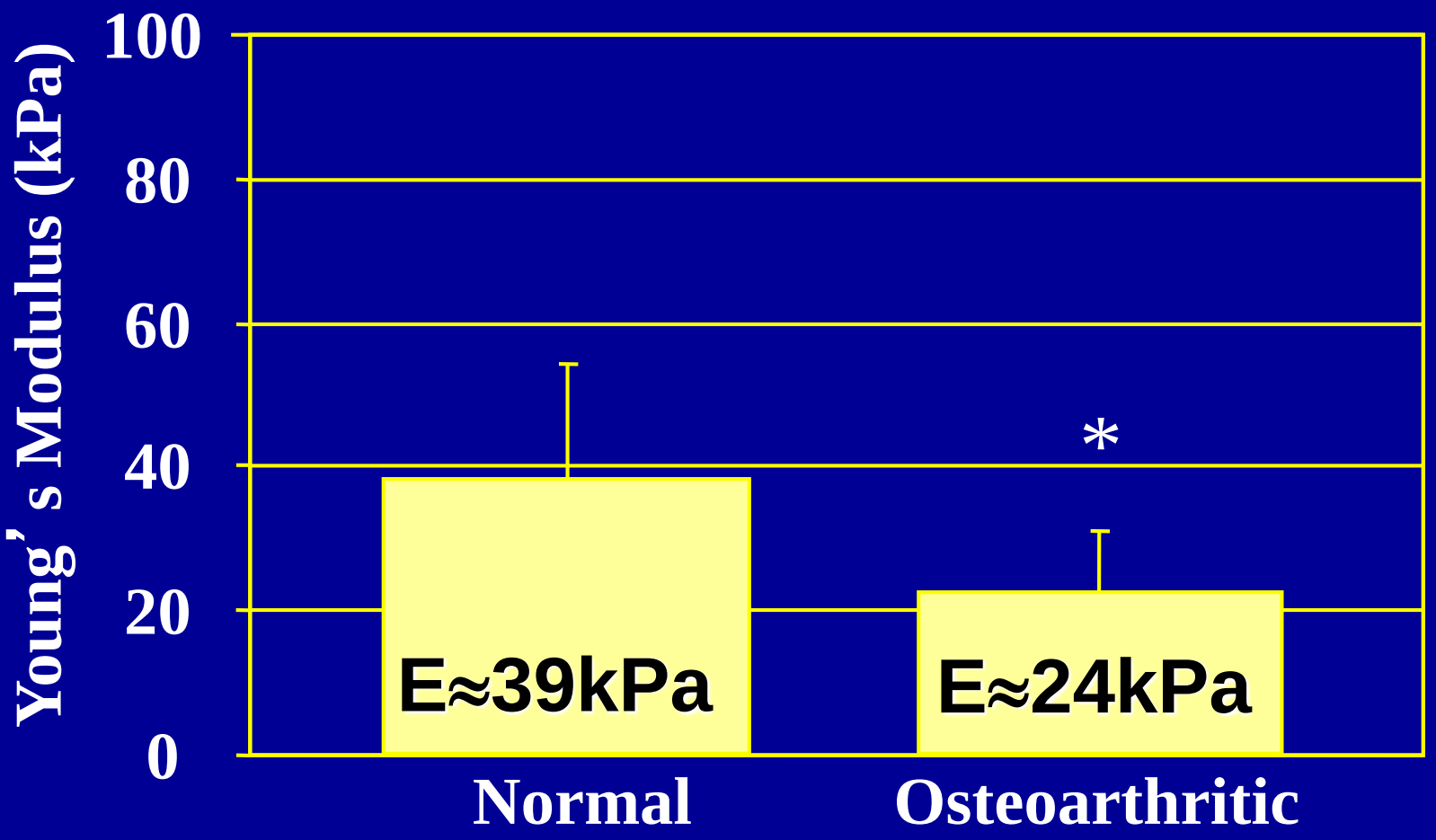
BIPHASIC MODEL



N>15 per group, **p< 0.0001, *p< 0.03 vs. the non-OA counterpart

Young's modulus is reduced with OA

BIPHASIC MODEL

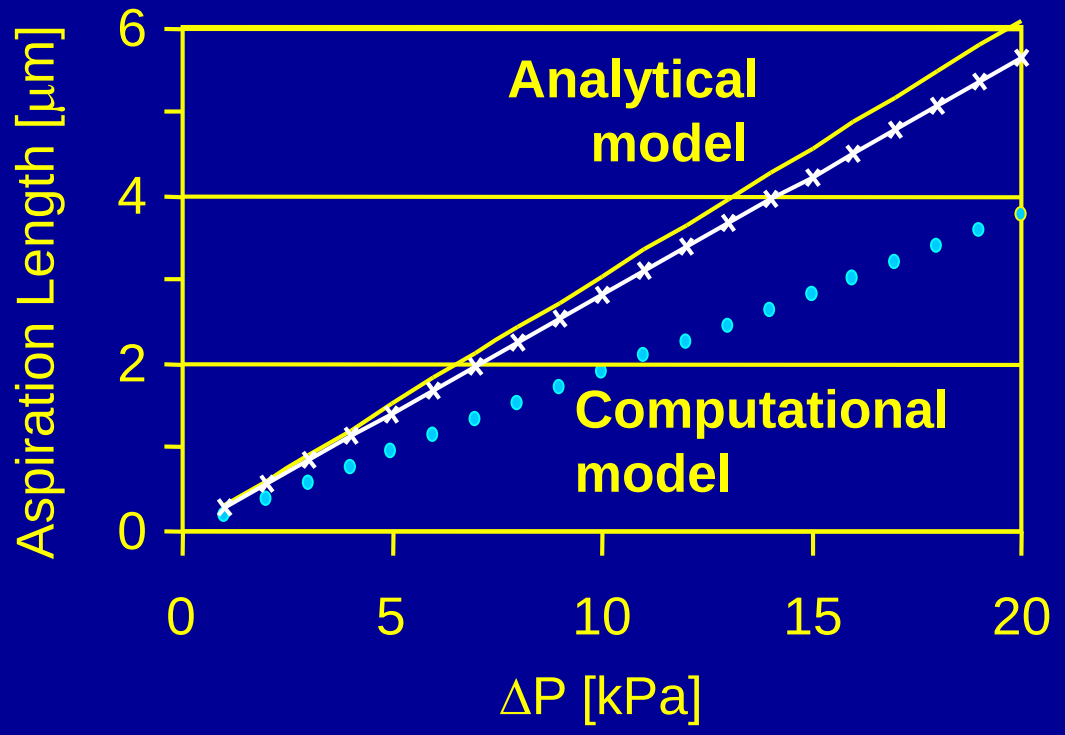


N>30 per group, *p<0.001 vs. the non-OA controls

Conclusions:

- **Both analytical and computational models fit well the data.**
- **The analytical model overestimates the Young's modulus by ~40%. Why?**

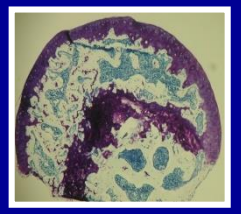
Because in the biphasic configuration part of the load is borne by the fluid flow.



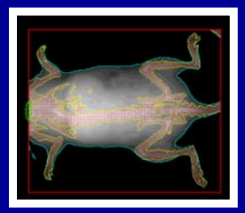
3 Materials and Methods

•Collagen type VI knockout mice were used

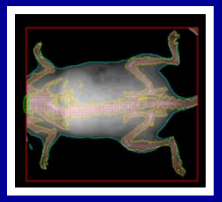
•The mechanical properties of the PCM from wild type and knockout mice were compared



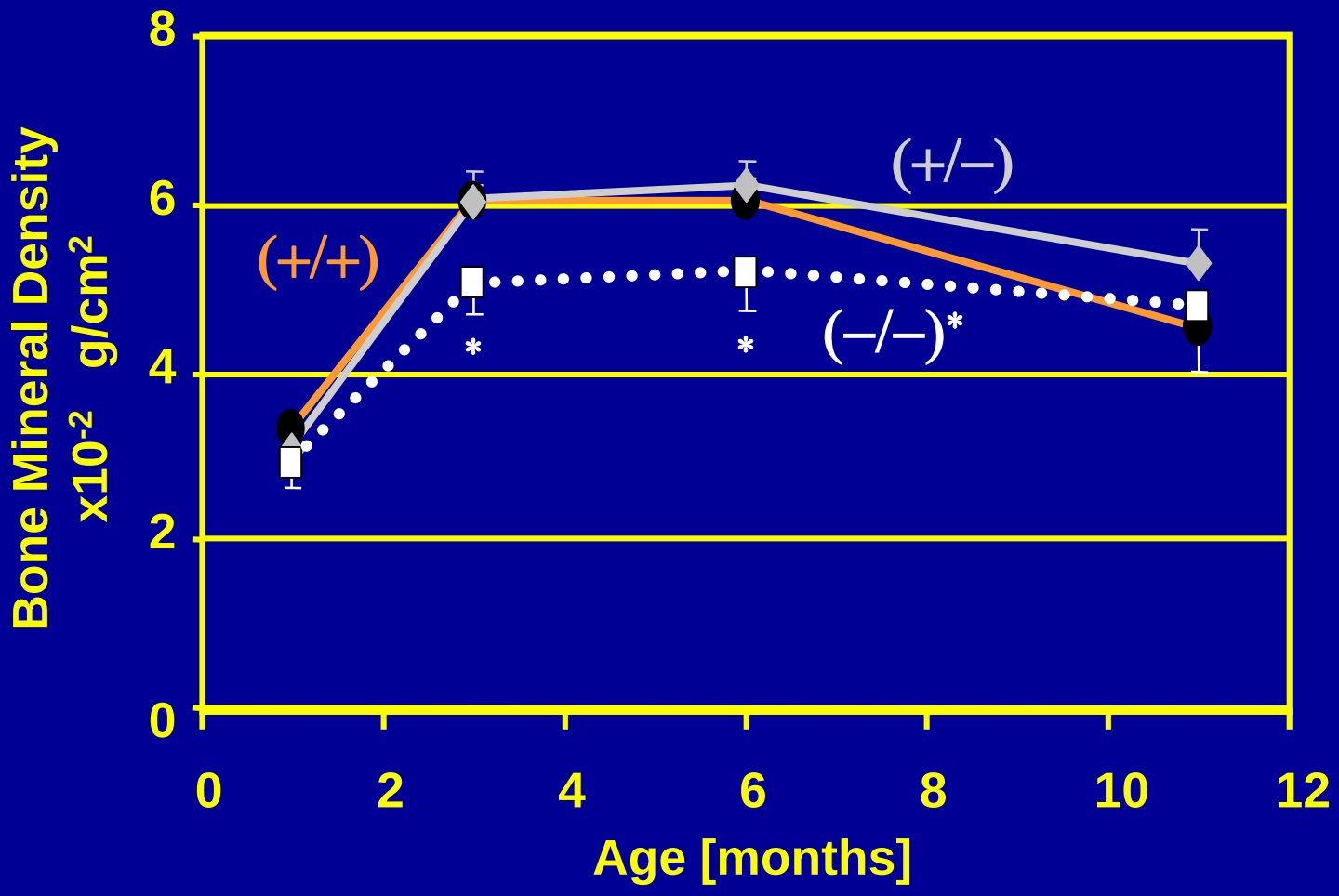
•Histological analysis of the femoral head was performed to quantify the development of cartilage and osteoarthritic changes



•X-ray absorptiometry was used to quantify bone mineral density



Bone mineral Density *



*p<0.0001, N=57

FUTURE AVENUES

Mechanical properties of soft tissues

- Examine time dependant behavior of soft tissues by developing a modified micropipette technique able to
  - Measure fluid flow upon application of aspiration pressure
  - Measure pressure upon application of a step displacement

  The advantage of this device will be the ability to uncouple the viscoelastic response from the biphasic response of the tissue.


Low risk


Quick turnover to start publishing


Not novel


Very similar to my current research


High competition

FUTURE AVENUES

Mechanotransduction + BioMEMS

- Construct a microfabricated device for in vitro application of mechanical loads on an array of cells incubated in an individually controlled chemical environment (i.e. growth factors and cytokines). Such devices will then be histologically processed to provide a clear picture on how the different chemical and mechanical environments affect cell phenotype .


Novel


Lots of applications


Something different than the current research on mechanobiology


Medium Risk


Needs time to be implemented

FUTURE AVENUES

Thermodynamic aspects of mechanotransduction

- Does energy from mechanical loading can be used to energize the cell?
  - Uniport one-way Channels can create chemical gradients using cell compression.

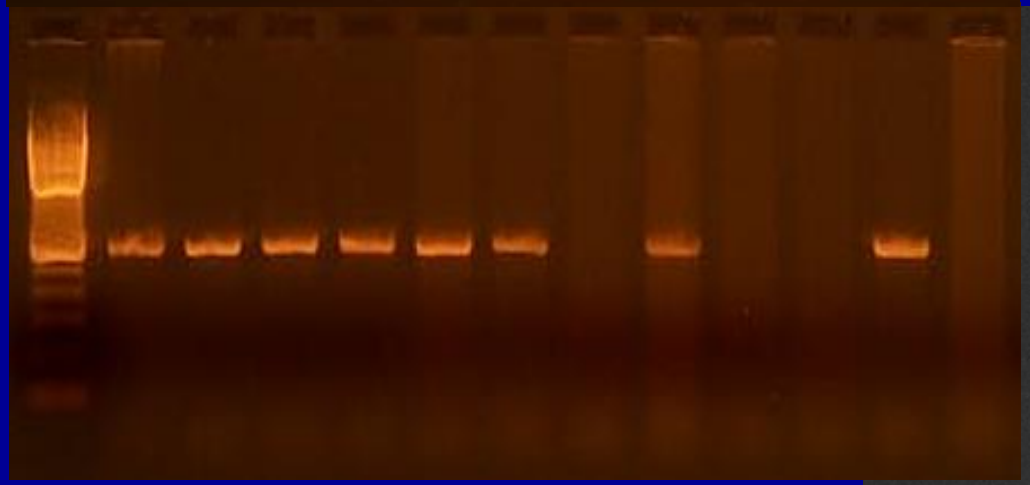

Very Novel / High Impact

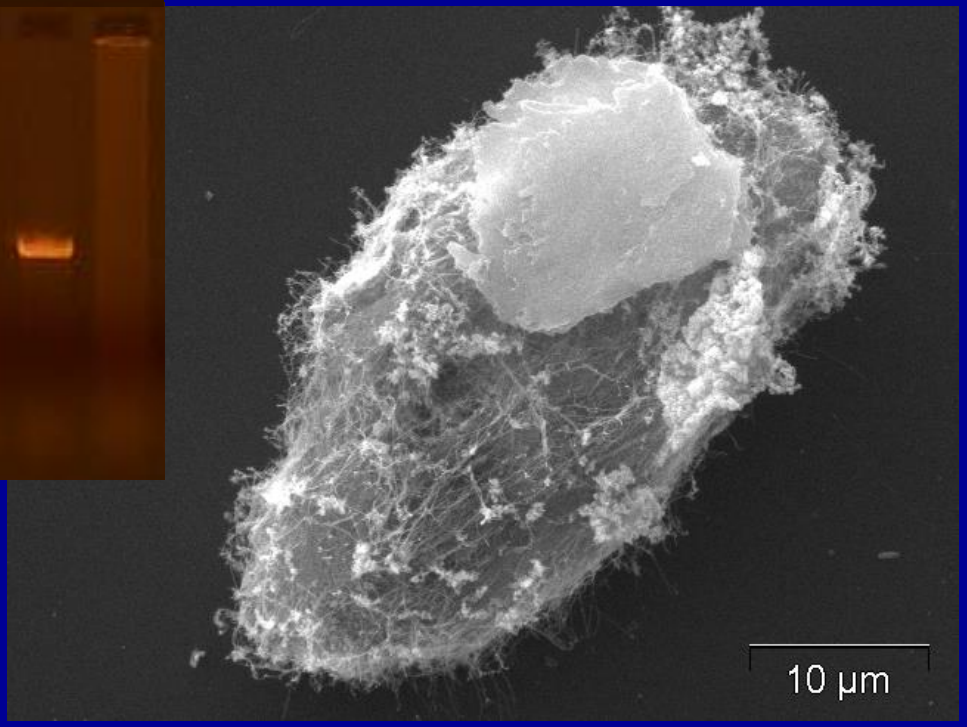

Different than current research on mechanobiology


Very risky


Requires long time to be implemented, Limited experience

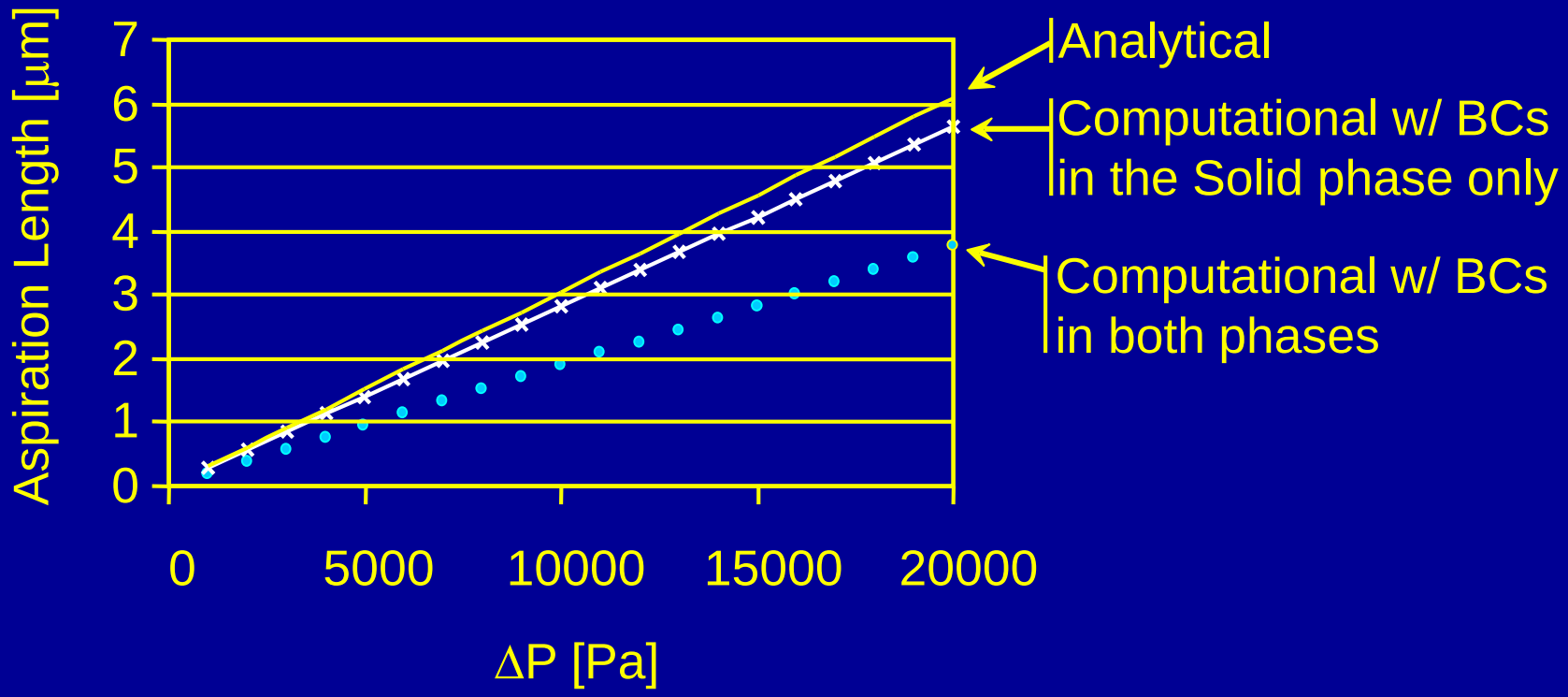

Requires good knowledge of ion channels



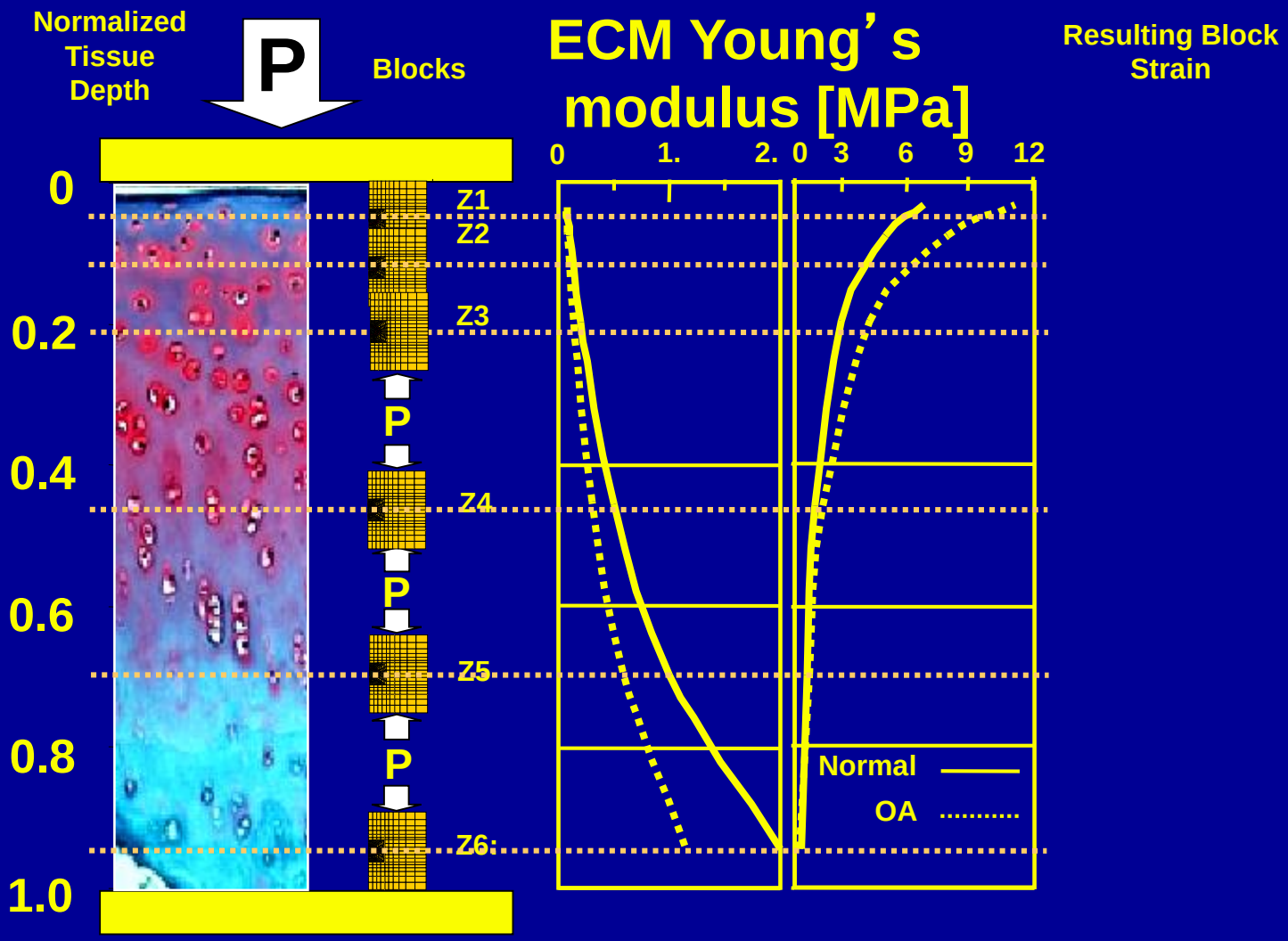

10 µm

Conclusions:

- **Both analytical and computational models fits well the data.**
- **The analytical model underestimates the Young's modulus by ~40% because in the biphasic model part of the load is borne by the fluid flow.**



2 | Finite Element Model



The Young's modulus distribution

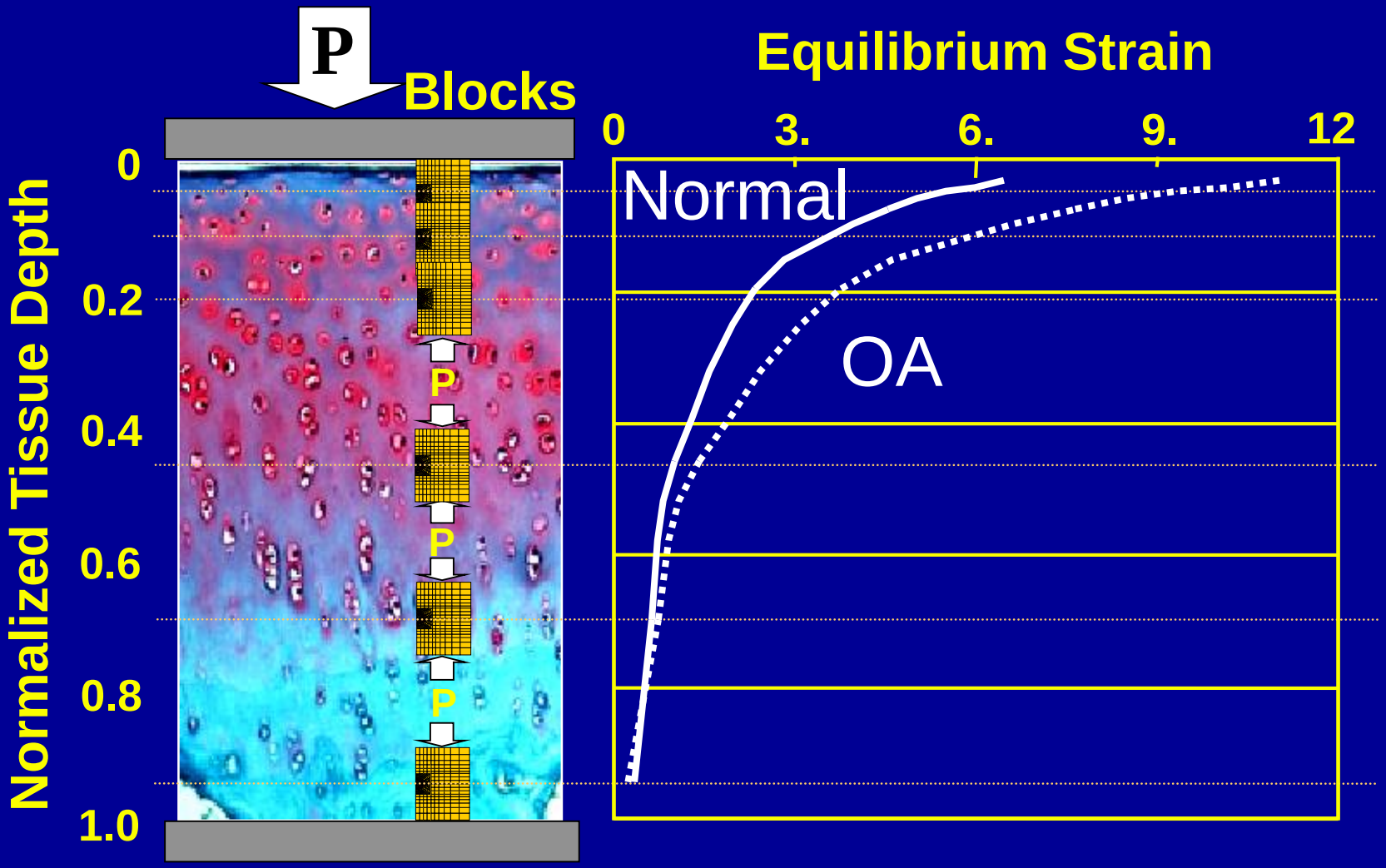
Koay et al., 2003, Knight et al., 2002, Trickey et al., 2000, Guilak et al 2000, Alexopoulos et al. 2004

2 | Material properties

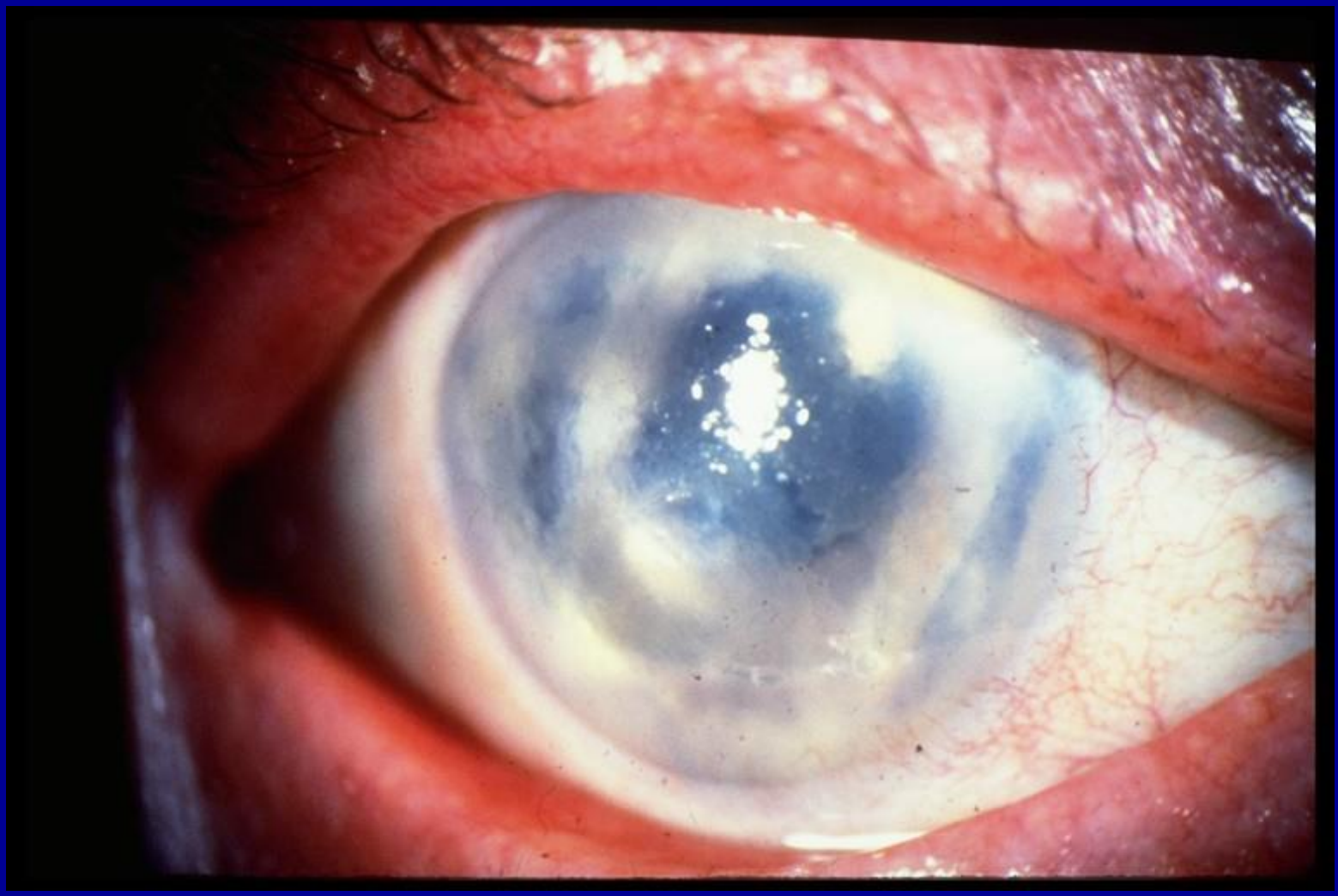
	PROPERTIES	CELL	PCM	ECM	
				S	M/D
Normal	Young's Modulus, E: kPa	0.36	40	100	1000
	Permeability, $k \times 10^{-15}$:m^4/Ns	4.2	0.04	1.0	1.0
	Poisson's ratio, *v*	0.4	0.04	0.04	0.04
OA	Young's Modulus, E: kPa	0.5	20	N/A	700
	Permeability, $k \times 10^{-15}$:m^4/Ns	2.4	0.13	N/A	2.0
	Poisson's ratio, *v*	0.4	0.04	N/A	0.04

Athanasiou K et al., 1995, Schinagl RM, et al., 1997, Trickey WR et al., 2000.
S: Superficial zone, M/D: Middle/Deep zone.

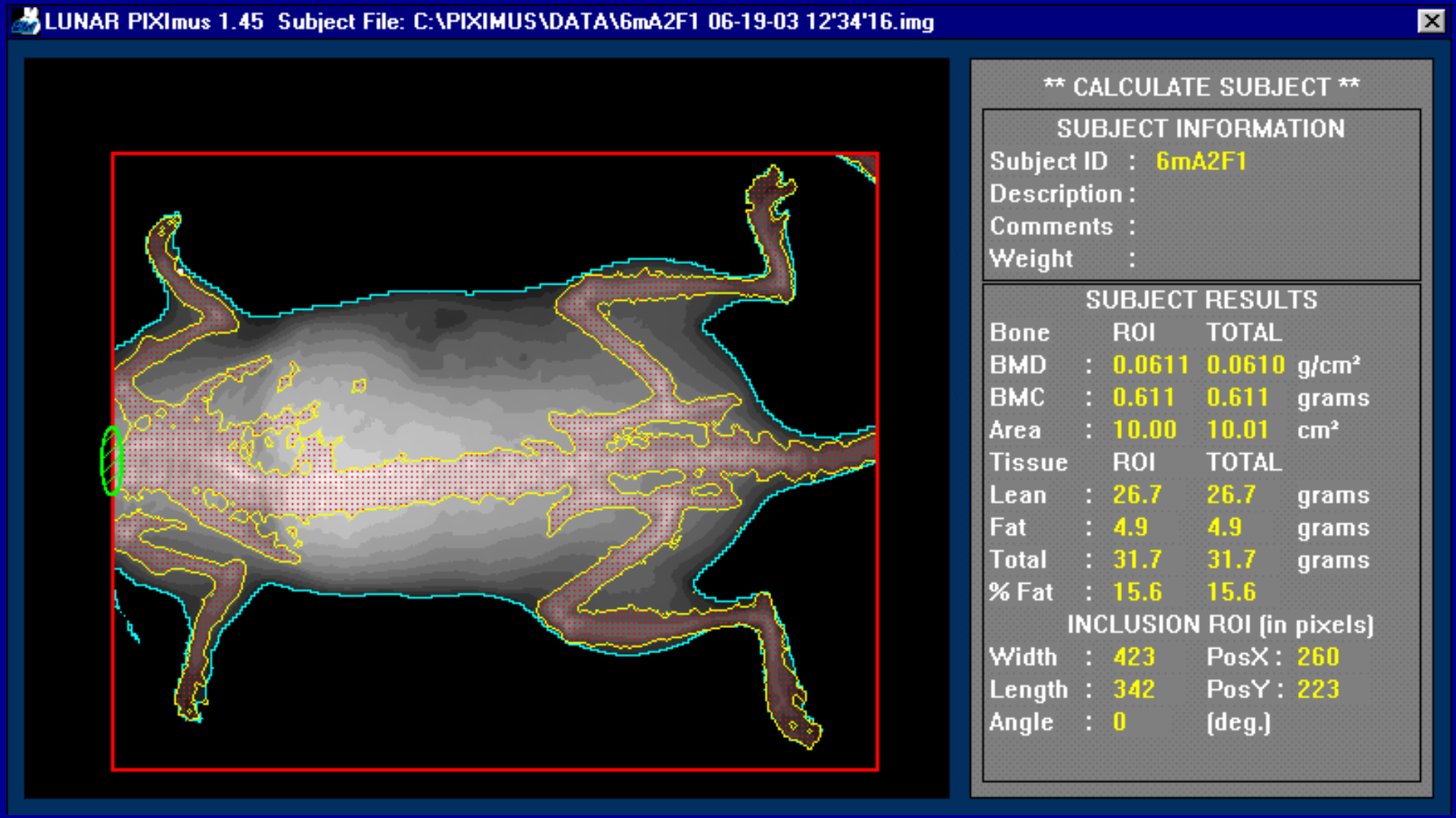
2 FEM: Compression tests



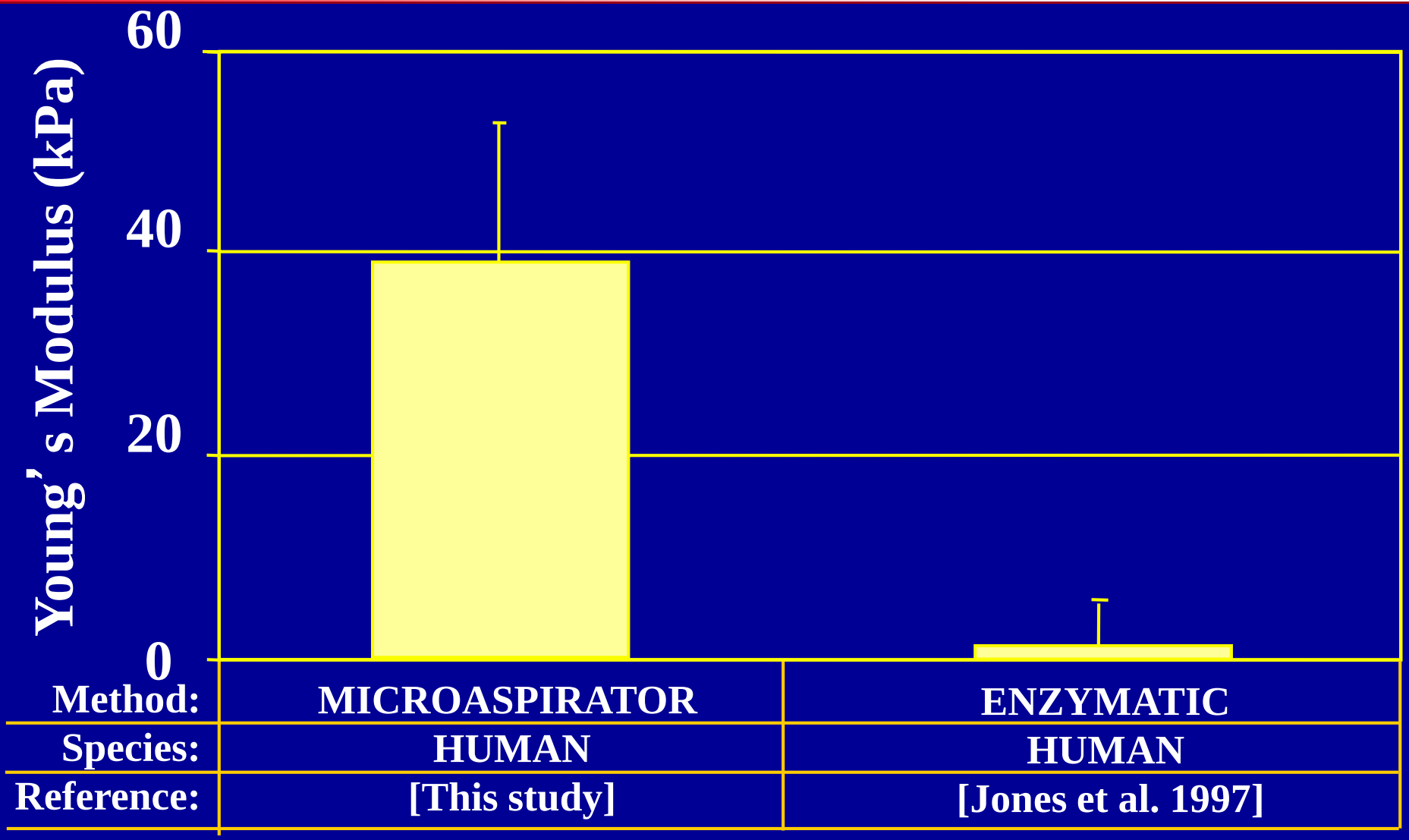
Bullous Keratopathy



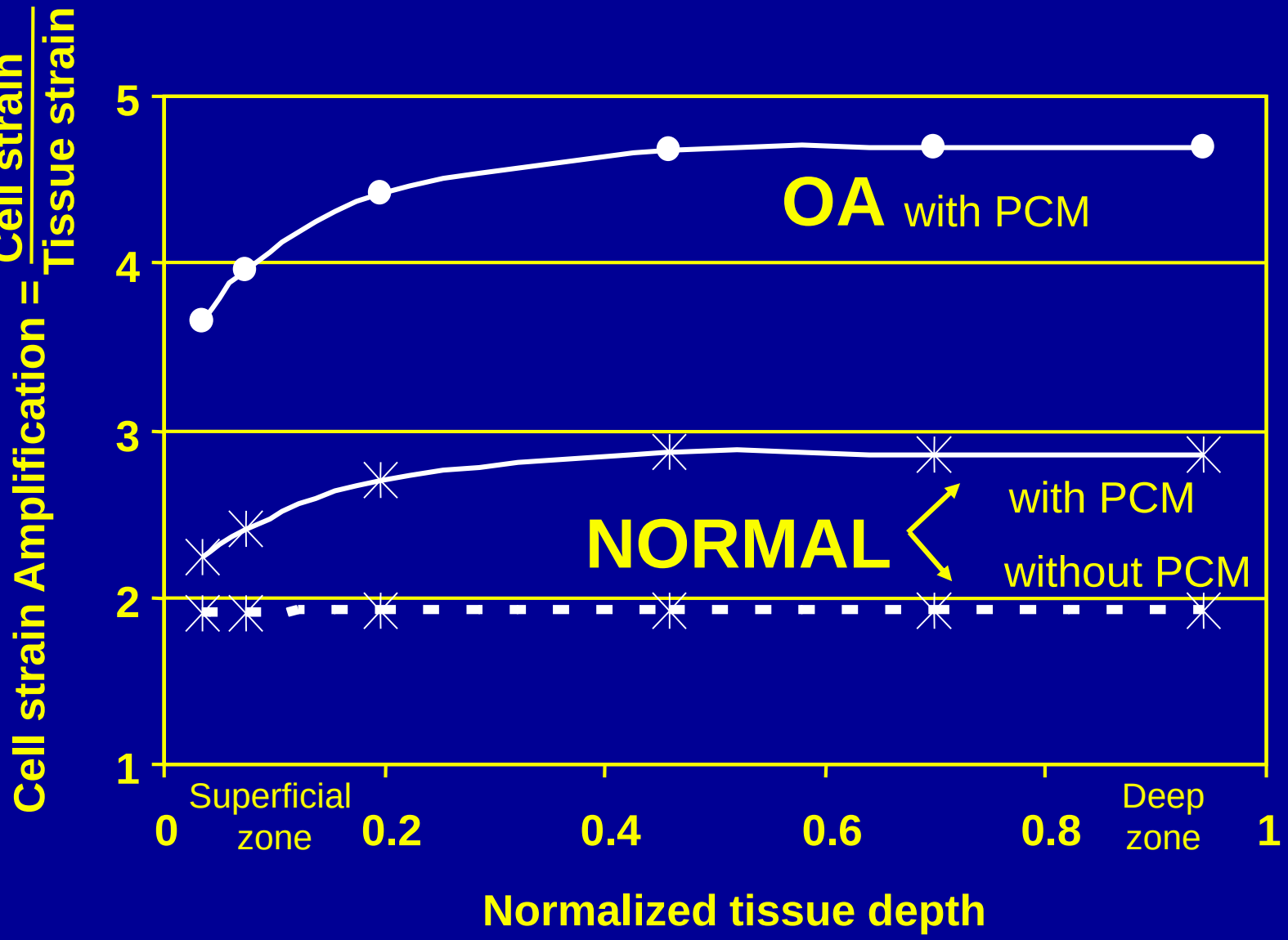
Dual energy X-Ray absorptiometry



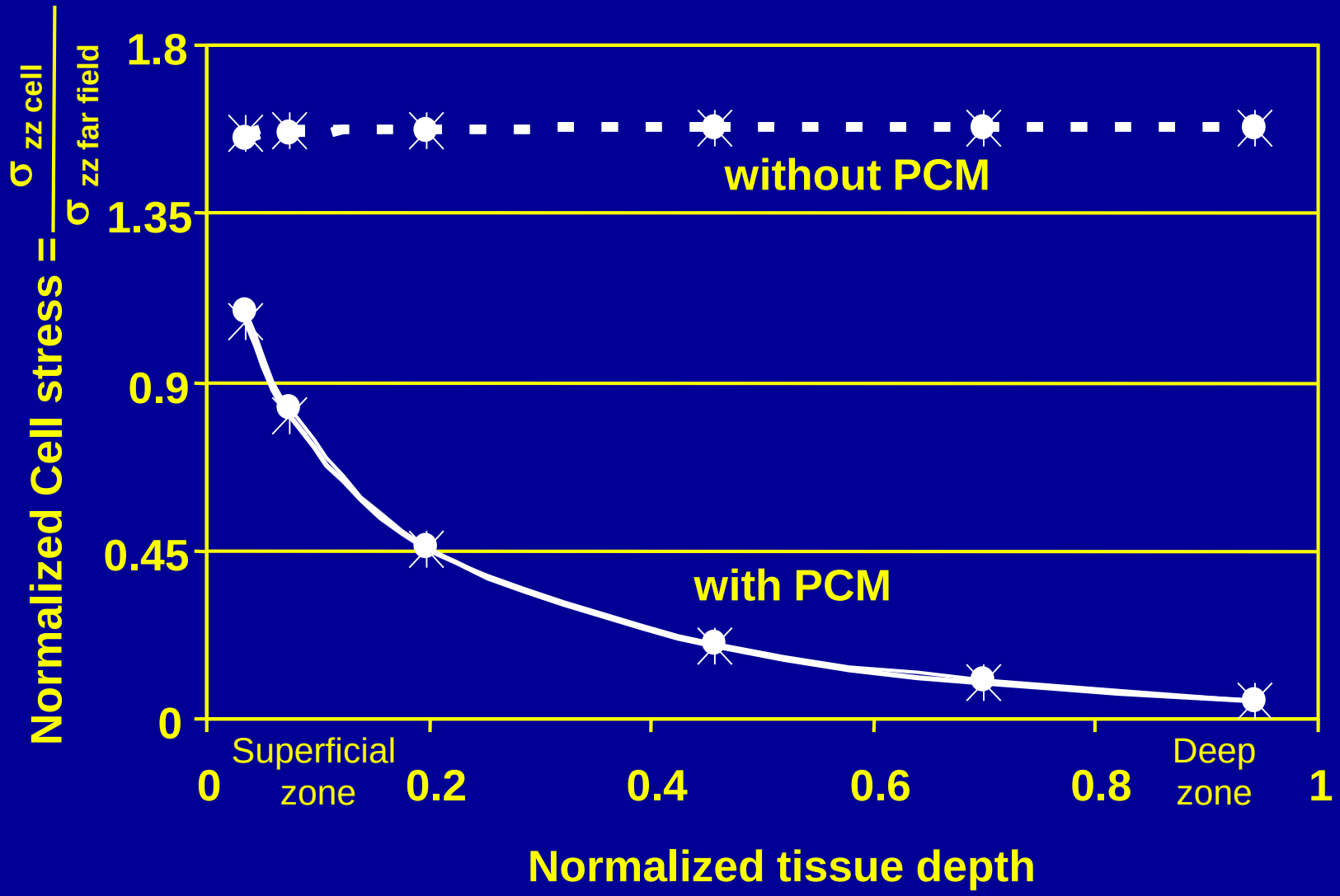
Conclusions: Enzymatic chondron isolation affects chondron stiffness



2 Results: Cell Strain

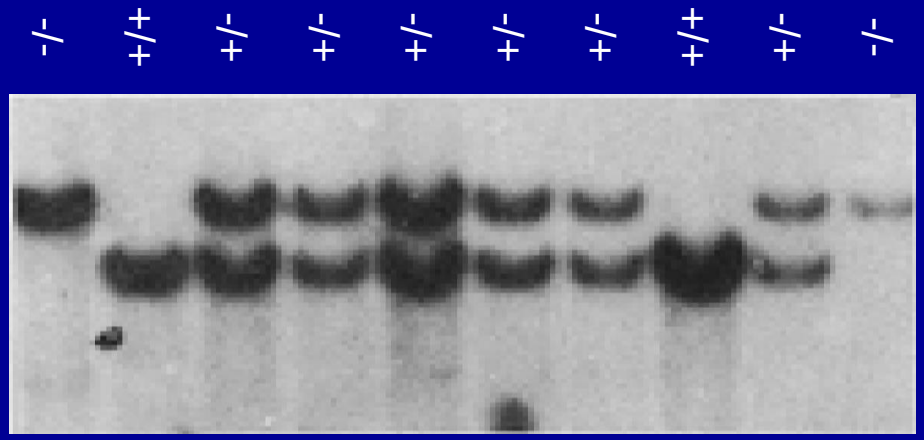


2 | Results: Cell Stress



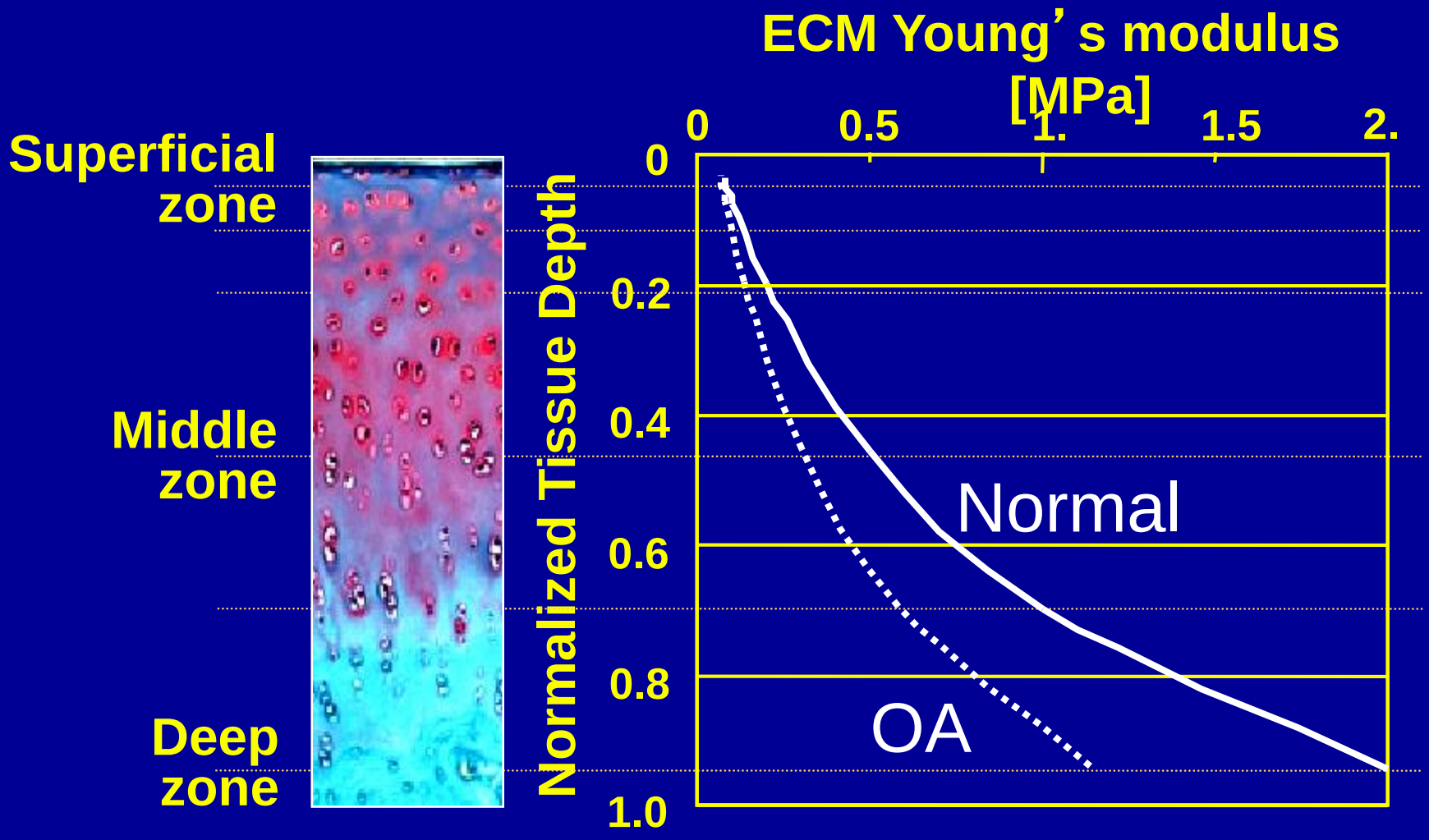
Collagen VI knockout mice

Bonaldo et al [1998] inactivated the col6α1 gene in mice. The homozygous mutant lacked Collagen VI.



Southern blot analysis of tail biopsies reveals insertion of the 1.2 kb neomysin-resistance cassette into the Col6α1 gene

2 Finite Element Model



Wang et al., 2002

3 Collagen VI and diseases

DOWNREGULATION
⇩
TISSUE LAXITY

- **Bethlem Myopathy**
  (Bonaldo et al. 1998, Speer et al. 1996)
- **Ullrich Congenital Dystrophy**
  (Higuchi et al. 2003, Niiyama et al. 2003, Pan et al. 1993)
- **Joint Laxity, Hypotonia**
  (Rauch et al. 1996)

UPREGULATION
⇩
TISSUE STIFFNESS

- **Fibrosis (Liver, Lung, Cardiac, Renal)**
  (Griffiths et al. 1992, Specks et al., 1995, Mollnau et al. 1995, Groma et al. 1998)
- **Bullous keratopathy***
  (Ljubimov et al. 1996)
- **Scar tissue (wound healing)**
  (Betz et al. 1993, Oono et al., 1993)
- **Scleroderma** (Takahashi et al. 1995)

Cartilage Degeneration

- **The most common type of cartilage degeneration is OSTEOARTHITIS**

- **Risk Factors include:**
  1. **Age**
  2. **Obesity**
  3. **Trauma**

E. B. Hunziker, *Osteoarthritis and Cartilage* (2001) **10,** 432–463

Cartilage Repair

Avascular, Aneural => Limited capacity for self repair

Therapeutic Interventions

1. **Lavage and Shaving** (cartilage washing, mechanical removal of diseased chondral tissue and smoothing of the cartilage surface)
2. **Bone Marrow Stimulation Techniques** (Abrasion / drilling leads to artificial bleeding to stimulate spontaneous tissue healing response)

E. B. Hunziker, *Osteoarthritis and Cartilage* (2001) **10,** 432–463

Cartilage Repair

3. Tissue transplantation

- Insertion of perichondrial/periosteal flaps in the focal cartilage defects reactivates tissue formation. Different cell sources can be used to fill the defect.
- Insertion of full thickness osteochondral grafts (mosaicplasty)

4. Total joint replacement

5. Cartilage tissue engineering

Conclusions….

Different computational models can be used to assess the role of the PCM in the micromechanical environment of chondrocytes